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CHEMISTRY OF URINE

CHEMISTRY OF URINE

A PRACTICAL GUIDE TO THE

ANALYTICAL EXAMINATION OF DIABETIC, ALBUMINOUS, AND GOUTY URINE

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PREFACE.

A CONSIDERABLE portion of the contents of this book was designed to form part of the concluding volume of my "COMMERCIAL ORGANIC ANALYSIS." Vol. III., Part 3rd, of that work deals mainly with substances of animal origin, and will complete the chief literary work of my life. But, of late, circumstances have led me to devote much attention to the chemical examination of urine, especially in relation to certain pathological conditions of great importance in Life Assurance reports, as well as in clinical diagnosis and prognosis. These examinations have caused me to investigate critically a large number of the analytical methods which are in vogue for the examination of urine, especially for sugar and albumin, and to confirm or disprove certain statements generally accepted as facts.

The results of this extensive laboratory work may be of assistance to many interested in Urinary Analysis. Physicians who are called on to advise as to the acceptance or rejection of candidates for Life Assurance often find this duty very onerous. Prognosis with regard to patients who may be suffering from glycosuria or albuminuria is a difficult and

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anxious task; and the more so as this task is one upon the performance of which the patient's immediate future is to cast so critical a light. Probably, also, there are analysts who will welcome this collection of analytical facts and methods.

While attempting to bring the majority of the tests and processes within the scope of everyday clinical diagnosis, or of the reports required for Life Assurance, I have also described other methods which cannot be applied except by those accustomed to analytical work, and who are possessed of the appliances of a well-appointed laboratory.

While desiring to give special prominence to the methods of examining diabetic, albuminous, and gouty urine, it appeared undesirable to omit all reference to subjects of collateral interest, such as the proportions of urea and total nitrogen in urine, the recent researches on creatinine and on xanthine derivatives, and the behaviour of urinary colouring matters. On the other hand, the book is not planned as a *complete* guide to Urinary Analysis. Thus, I have omitted all mention of the methods of determining phosphates and most of the other mineral constituents of urine; firstly, because they are not of great pathological interest, and secondly, because I have nothing to say about them which cannot be found in every physician's and analyst's library. Should this production meet with such a reception as to call for the issue of a second edition, it may be desirable to supply this and other omissions.

It is with great pleasure that I acknowledge the valuable assistance rendered me by Dr James Edmunds,

of Dover Street, Piccadilly, to whom I am greatly indebted for the perusal of the whole of the proofs, and for many valuable suggestions, both scientific and literary. I am also obliged to Messrs A. W. Gerrard, F. G. Hopkins, and G. Stillingfleet Johnson for the perusal and correction of particular proof-sheets. Much time and labour have been devoted in my laboratory to the examination of many of the tests and processes described, and my cordial thanks are due to Mr G. Bernard Brook and Mr Arnold R. Tankard for their zealous and painstaking assistance in this arduous work.

The progress of further investigations which I have in hand would be materially facilitated by increased opportunities of examining abnormal specimens of urine. I therefore take this opportunity of soliciting the co-operation of clinical workers, who would greatly oblige me by forwarding for examination specimens of any urines which appear to justify such a course. There is reason to believe that some of the less known constituents of urine, such as glycuronic acid and its compounds, creatinine, xanthine, the indoxyl and skatoxyl derivatives, and particular pigments, are greatly augmented under certain pathological conditions at present not fully understood, and the systematic examination of urines of abnormal character would probably materially extend our knowledge of this difficult and obscure subject. In sending such samples, I would request that the urine be poured into a clean, strong eight-ounce bottle, which should then be at once securely corked, carefully packed, and distinctly labelled, with the date and hour of passing, and with

the name and address of the sender. The sample should be forwarded at once by parcel-post, and full information as to the patient's history should be sent to me at the same time by letter-post. Of course the complete examination of urine for abnormal constituents cannot be effected on so small a quantity as eight ounces, but this amount will suffice to ascertain whether a more extended analysis of urine from the same source is desirable.

ALFRED H. ALLEN.

67, SURREY STREET,
SHEFFIELD, *June 1st*, 1895.

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GENERAL COMPOSITION OF URINE.

URINE is an excretion of extremely complex composition, even in a state of health, while pathological urine contains a variety of other bodies absent from or existent only in traces in the normal excretion.

W. D. Halliburton gives the following list of constituents, the arrangement being based on Hoppe-Seyler's classification :—

1. *Urea and related substances.* Urea, uric acid, allantoin, oxalic acid, xanthine, guanine, creatinine, thiocyanic (sulphocyanic) acid.

2. *Fatty and other non-nitrogenous substances.* Fatty acids of the series $C_nH_{2n}O_2$; oxalic, lactic, and glycero-phosphoric acids; minute quantities of certain carbohydrates.

3. *Aromatic substances.* The ethereal sulphates of phenol, cresol, pyrocatechin (catechol), indoxyl, and skatoxyl; hippuric acid; aromatic oxy-acids.

4. *Other organic substances.* Pigments, ferments (especially pepsin), mucus, humous substances; and in dogs, cynurenic and urocanic acids.

5. *Inorganic compounds.* Sodium and potassium chlorides; potassium sulphate; sodium, calcium and

magnesium phosphates; silicic acid; calcium carbonate; ammonia salts.

6. *Gases.* Nitrogen and carbonic acid.

In addition to the above constituents of normal urine, there may be present in certain pathological conditions :—albumin and other proteids, hæmoglobin, methæmoglobin, bile-pigments, bile-acids, abnormal urinary pigments, leucine and tyrosine, oxymandelic acid, dextrose, milk sugar, glycuronic acid, fats, lecithin, cholesterin, cystin; constituents derived from food or drugs; and organised bodies, such as blood-corpuscles, urinary casts, and renal epithelium.

Several other urinary constituents of considerable importance are omitted from Halliburton's list. Among these may be named the acetone, aceto-acetic ether, and hydroxy-butyric acid often present in diabetic urine during the latter stages of the disease; hypoxanthine; and substances resulting from the imperfect metabolism of food or tissue.

The specific gravity of urine is roughly a measure of the contained solids.

The constituents of urine of chief importance are the urea, uric acid, and phosphates, and it is to the determination of these that the quantitative examination of healthy urine is generally limited.

Constituents of secondary importance are the creatinine, hippuric acid, chlorides, and sulphuric acid in the two forms of metallic sulphates and ethereal salts.

In healthy urine, sugar and albumin exist only in traces, if at all; but in typical diabetic urine a variety of sugar is the leading constituent. Albumin sometimes co-exists with sugar in diabetic urine, but its presence is more characteristic of certain other diseases.

The urine passed in twenty-four hours by a man

weighing 66 kilogrammes (= 144½ lbs.) is stated by Parkes to measure 1500 c.c. (52 oz.), and to contain about 72·5 grammes (or 2½ oz.) of solids, having the following composition :—

	Percentage Composition of Solids.	Absolute Weight of Solids in Grammes.	Weight per 1000 of Body Weight.
Urea, CH_4N_2O,	45·75	33·18	0·5000
Creatinine, $C_4H_7N_3O$,	1·25	0·91	0·0140
Uric acid, $C_5H_4N_4O_3$,	0·75	0·55	0·0084
Hippuric acid, $C_9H_9NO_3$,	0·55	0·40	0·0060
Pigment and other organic substances,	13·79	10·00	0·1510
Sulphuric acid, SO_3,	2·77	2·01	0·0305
Phosphoric acid, P_2O_5,	4·36	3·16	0·0480
Calcium,	0·35	0·26	0·0004
Magnesium,	0·28	0·21	0·0003
Potassium,	3·45	2·50	0·0420
Sodium,	15·29	11·09	0·1661
Chlorine,*	10·35	7·50	0·1260
Ammonia,	1·06	0·77	0·0130
	100·00	72·54	1·1057
*= Sodium Chloride,	17·04	12·36	0·1852

According to J. L. W. Thudichum (*Pathology of the Urine*, 1887), the average volume of urine excreted in twenty-four hours, by men weighing from 60 to 65 kilogrammes, is from 1400 to 1600 c.c. (48 to 56 fluid ounces), of a specific gravity averaging 1·020. The solids contained in this quantity of urine range from 55 to 66 grammes, and are stated to be composed as follow :—

Urea,	30 to 40	grammes.
Creatine,	0·30	
Creatinine,	0·45	0·75 „
Xanthine and its derivatives,	undetermined.	
Uric acid,	0·50	„
Hippuric acid,	0·50	„
Cryptophanic acid,	0·65	„

Colouring matters,	undetermined.
Biliary acids,	0·012 grammes.
Oxalic, oxaluric, and nitrogenised derivative of sarco-lactic acid,	undetermined.
Acetic acid,	0·288 ,,
Formic acid,	0·05 ,,
Carbonic acid,	undetermined.
Sulphuric acid,	1·5 to 2·5 ,,
Sulphur in other forms,	0·20 ,,
Phosphoric acid as alkaline phosphates,	3·66 ,,
Earthy phosphates,	1·28 ,,
Lime,	0·17 ,,
Magnesia,	0·19 ,,
Iron,	undetermined.
Potassium and sodium chlorides,*	10 to 13 ,,
Potash and soda,	undetermined.
Ammonia,	0·7 ,,
Methylamine,	trace.
* Containing chlorine,	6 to 8 ,,

Yvon and Berlioz have published the results of numerous analyses of normal urine (*Lancet*, ii., 1888, page 629). Their mean figures are as follow :—

	Male.		Female.	
	Per litre.	Per diem.	Per litre.	Per diem.
Specific gravity,	1·0225		1·0215	
Volume,		1360 c.c.		1100 c.c.
Urea,	21·5 grms.	26·5 grms.	19·0 grms.	20·5 grms.
Uric acid,	0·5 ,,	0·6 ,,	0·55 ,,	0·57 ,,
Phosphoric acid,	2·5 ,,	3·2 ,,	2·4 ,,	2·6 ,,

With the exception of the uric acid, which is almost the same for the two sexes, the amounts under each head are higher for males than for females. The proportion of urea to uric acid is 40 : 1, and that of urea to phosphoric acid 8 : 1.

W. Camerer (*Zeit. Biol.*, xxiv. 306) has recorded the amount of total nitrogen contained in normal urine, and has compared it with that eliminated in the form of urea. Thus the mixed urine from a

number of persons measured on the average 1840 c.c., had a specific gravity of 1·016, and contained :—

	Per diem.	Per cent.
Total nitrogen, . . .	16·06 grammes,	0·873
Nitrogen as urea, . . .	14·15 „	0·769
Nitrogen in other forms, . .	1·91 „	0·114

The nitrogen passed in the form of urea is about 90 per cent. of the total.

The foregoing analyses probably fairly represent the average composition of urine, but they take no account of the variation in composition resulting from change of food. The two following analyses by G. Bunge furnish interesting information in this connection. They were carried out on the urine of a young man in good health, who was fed in succession on animal and vegetable diet. The urine was collected on the second day, after an exclusive diet of roast beef, with a little salt and spring water. In the second case the urine was also collected on the second day, after an exclusive diet of wheat-bread, butter, a little salt, and spring water. Bunge points out that these analyses are probably unique among those published, in that all the important constituents were determined on the same sample of urine. The following were his results :—

	Meat Diet.	*Bread Diet.*
Total measure of urine in 24 hours, . .	1672 c.c.	1920 c.c.
Urea, . . .	67·2 grammes.	20·6 grammes.
Creatinine, . .	2·163 „	0·961 „
Uric acid, . .	1·398 „	0·253 „
Sulphuric acid, .	4·674 „	1·265 „
Phosphoric acid, .	3·437 „	1·658 „
Lime, . . .	0·328 „	0·339 „
Magnesia, . .	0·294 „	0·139 „
Potash, . . .	3·308 „	1·314 „
Soda, . . .	3·991 „	3·923 „
Chlorine, . .	3·817 „	4·996 „

The figures for sulphuric acid were obtained by boiling the urine with hydrochloric acid and barium chloride, and hence include both that existing as metallic sulphates and that present as ethereal salts.[1]

It will be observed that the urea, creatinine, and uric acid are all greatly increased by a meat diet. The phosphoric and sulphuric acids, which are largely derived from the oxidation of albuminoids, also show a marked increase.

[1] In the foregoing analyses the sulphuric acid and chlorine are sufficient to convert all the inorganic bases into sulphates and chlorides. The ammonia was not determined, but if present in normal proportion (*i.e.*, from 0·4 to 0·9 gramme), it would suffice to convert the phosphoric acid into AmH_2PO_4. Further, the saturating power of the sulphuric acid is over-estimated, since some of it existed in the form of phenyl-sulphuric acid, $C_6H_5.HSO_4$, or allied compounds, exerting only a mono-basic function. The quantity of sulphur in the form of ethereal salts in human urine averages one-tenth of that present as metallic sulphates. On acidulating the urine with acetic acid, and adding barium chloride, the metallic sulphates are precipitated. If the liquid be filtered, and the filtrate rendered strongly acid by hydrochloric acid and boiled, the conjugated sulphuric acid salts are broken up, and the resultant sulphuric acid can then be thrown down as barium sulphate. If the liquid be again filtered, evaporated to dryness, and the residue fused with nitre, an additional quantity of sulphuric acid is formed, corresponding, in human urine, to 10–20 per cent. of the total sulphur excreted, but rising in anomalous cases to a larger proportion. This unoxidised sulphur exists in the urine partly in the form of cystin, a body having the composition $C_3H_7NSO_2$; but a portion is excreted in the form of thiocyanates. The ethereal sulphates are represented by the potassium salts of phenyl-sulphuric acid, $C_6H_5.HSO_4$, indoxyl-sulphuric acid, $C_8H_6N.HSO_4$ (the so-called urinary indican), skatoxyl-sulphuric acid, $C_8H_5(CH_3)N.HSO_4$, and similar derivatives of catechol (pyro-catechin) and quinol (hydroquinone.)

PRELIMINARY EXAMINATION OF URINE.

VOLUME.—The quantity of urine passed by a healthy man in twenty-four hours is commonly stated at about 50 oz. (1450 c. c.), but the observations on which this statement is founded have been chiefly made on the inhabitants of gaols, workhouses, or barracks, where the inclination and opportunity for drinking is limited. Many men in a state of perfect health habitually pass a considerably larger volume than 50 oz. daily. An excessive excretion indicates polyuria, and is a very common, but not invariable, accompaniment of glycosuria.

Whenever practicable, all observations on urine should be made on portions of the mixed excretion of twenty-four hours, otherwise very misleading conclusions may be formed. Thus the acidity, specific gravity, colour, and other characters of urine vary greatly with the period of the day and the time which has elapsed since the last meal. In cases of diabetes of a mild type, or which are progressing towards recovery, sugar is often nearly or entirely absent from the morning urine, but returns for several hours after partaking of a meal of which carbohydrates have formed a portion.

APPEARANCE.—The *colour* of normal urine is pale yellow or amber. Typical diabetic urine is very pale, but this peculiarity appears to be due to its greater volume and consequent dilution of the urinary

pigments, as compared with the normal excretion, rather than to actual diminution of colouring matter.

Dark urines owe their colour to bile-pigments, blood, tinctorial drugs (*e.g.*, rhubarb), or to excess of the pigments present in normal urine. The subject is discussed more fully in a subsequent section.

URINARY SEDIMENTS. — Normal human urine is clear, or contains a fine flocculent precipitate of mucus. The urine of herbivorous animals is turbid when excreted, from the separation of earthy phosphates, and human urine is not unfrequently passed in the same condition. In certain diseases very turbid urine is voided, and may contain mucus, pus, tube-casts, epithelium, chyle, blood, &c.

However clear the urine may appear when freshly passed, a deposit almost always becomes visible on allowing it to stand for a few hours. In normal urine this deposit is nearly transparent, very light and flocculent, and consists of *mucus*. A heavy pulverulent deposit, of a buff or fawn colour, generally consists of *urates;* but *earthy phosphates* are not unfrequently deposited from urine of an alkaline reaction. A deposit of urates disappears on heating, while one consisting of phosphates is permanent, and often becomes denser on boiling the urine.

On standing for several days, normal urine deposits the whole, or at any rate the greater part, of its uric acid in the form of acid urate of sodium. On still further standing, the urine becomes alkaline from the decomposition of the urea with formation of ammonium carbonate.

ODOUR.—The smell of urine is peculiar and slightly aromatic. The odour of the normal excretion is said to be due to phenol, and taurylic and damoluric acids. The odour of diabetic urine often recalls that of hay, but in the later stages of the disease the smell of acetone is frequently perceptible. When cystin is

present the smell of the urine is at first like that of sweet-briar, but afterwards becomes offensive. Turpentine produces an odour like that of violets, and the essential oils of cubebs, copaiba, santal-wood, &c., when taken internally, communicate characteristic smells to the urine. On long keeping, or when it has undergone fermentation in the bladder, urine has an ammoniacal odour, while an excretion containing blood or pus is often putrid, and occasionally evolves sulphuretted hydrogen.

TASTE.—The taste of urine is said to be at once salt and bitter. Diabetic urine has a distinctly sweet taste, owing to the presence of sugar, the existence of which was first recognised by this character. The French chemists still lay stress on the "saveur" as a test for urine.

REACTION OF URINE.—Normal urine has commonly a marked acid reaction to litmus, but during the so-called "alkaline tide" which follows meals the urine has not unfrequently a distinct alkaline reaction. Diabetic urine is very generally acid, and the reaction is not readily altered by food or alkaline medicines.

The acid reaction of urine has been attributed to the presence of traces of hippuric or other acids in an uncombined state, but is in all probability due to the occurrence of sodium dihydrogen phosphate (acid phosphate of sodium), NaH_2PO_4, produced by the reaction of the uric and sulphuric acids resulting from the metabolysis of the food on the basic sodium phosphate of the blood. Hence it follows that the acidity of urine is increased by muscular exercise and the consumption of highly albuminous food, as of course by the internal administration of mineral acids.

Normal urine becomes alkaline after a vegetable diet containing potassium salts of organic acids. Thus the acid potassium salts of tartaric, citric, malic, and

other vegetable acids are largely present in acid fruits, and on combustion are converted into potassium carbonate. Hence the urine passed after a fruit diet has a strong alkaline reaction and effervesces on addition of a mineral acid. Potatoes cause the urine to be strongly alkaline, because they contain but a small proportion of proteids, and hence yield but little sulphuric acid on oxidation; while on the other hand they are rich in potassium malate, which is converted into carbonate on ignition. The cereals and leguminous seeds, on the contrary, yield a urine as acid as that excreted under a meat diet, since they are rich in both albuminoids and phosphates.[1]

The *alkalinity* of urine may be due to the presence of carbonates or basic phosphates of the alkali-metals, or to the presence of carbonate of ammonium. This last body is derived from the hydrolysis of the urea, according to the following equation :— $CH_4N_2O + 2H_2O = (NH_4)_2CO_3$. This change occurs spontaneously in urine when kept for some days, and is owing to the action of a ferment called *torula ureæ*. If any such ferment find access to the bladder, as by the use of a septic catheter, decomposition may occur before the urine is voided, and the excretion may have an alkaline reaction to litmus even when freshly passed. The nature of this alkalinity may be readily ascertained by moistening red litmus paper with the urine. If the alkalinity be due to ammonia, the blue colour will disappear as the paper dries, but otherwise will be permanent.

The quantitative determination of the *acidity* of urine may be made by titrating 100 c.c. of the sample

[1] Reasoning from these facts, F. Bunge regards cheese as a particularly unsuitable food for persons inclined to gravel, since in its manufacture the alkaline salts are largely lost in the whey, while the casein yields much sulphuric and phosphoric acid on combustion. Salted meat and fish he considers objectionable for similar reasons.

with decinormal caustic soda, using phenolphthalein or alizarin as an indicator. The addition of the alkali is continued till a deep red colour is obtained with the former indicator or a violet with the latter. Each 1 c.c. of standard alkali required represents 0·012 gramme of sodium dihydrogen phosphate, NaH_2PO_4, to which salt the acidity of normal urine is very probably due.

Similarly, according to Freund and Toepfer,[1] the *alkalinity* of urine can be determined by titrating 100 c.c. with decinormal sulphuric or hydrochloric acid, the end-reaction being the point at which the red colour due to phenolphthalein disappears, or the violet of alizarin changes to yellow. Each 1 c.c. of decinormal acid required is stated to represent, when the former indicator is used, 0·0106 gramme of sodium carbonate, Na_2CO_3; or 0·0164 of trisodium phosphate, Na_3PO_4. When alizarin is the indicator used, each 1 c.c. of acid represents half these quantities.

Whatever variations in the reaction of urine may be observed hourly under the influence of food, &c., the mixed excretion of twenty-four hours, if of normal character, will invariably be found to exhibit an acid reaction.

Specific Gravity and Total Solids of Urine.—The specific gravity of normal human urine varies from 1015 to 1025, averaging about 1020; but after great physical exertion and consequent profuse perspiration it has been known to be as high as 1035 in the case of healthy persons. On the other hand, after excessive drinking it has fallen as low as 1002. Typical diabetic urine is of very high specific gravity, usually exceeding 1030 and occasionally rising as high as 1074.

The specific gravity of urine is most conveniently ascertained by means of the hydrometer, an instrument which, if accurately constructed, gives, with reasonable care, indications quite delicate enough for

[1] *Zeit. physiol. Chem.*, xix. 84, and *Jour. Chem. Soc.*, lxvi. part ii. page 260.

ordinary purposes. It must be remembered, however, that the readings are very materially affected by alterations of temperature. Hence the urine should be brought as nearly as possible to a temperature of 60° F. (= 15·5° C.), and on no account should the hydrometer reading be taken when the urine is sensibly warm.[1]

The specific gravity of urine is roughly an indication of the proportion of solid matter contained in it, and hence of the total waste of the system. It would be an accurate measure of this if the solids were of a homogeneous nature, but as solutions of equal strength of sodium chloride, urea, sugar, and other urinary constituents have different specific gravities, the density of the urine will vary with the *nature* of the contained solids, as well as with their *amount*.

The following are the specific gravities at 60 F. (= 15·5 C.) of solutions of urinary constituents containing 10 per cent. of the solids :—

Substance.	*Specific Gravity.*	*Observer.*
Sodium Chloride (common salt),	1073·35	Gerlach.
Urea, ...	1028·2	A. H. Allen.
Dextrose, ...	1040·0	F. Salomon.
Albumin, ...	1026·0	——

Hence to deduce accurately the percentage of solids from the gravity of urine it would be necessary to ascertain the proportion of each of the leading constituents; but when that was effected, the specific gravity would no longer have any interest.

If the specific gravity of a sample of urine above that of water (= 1000) be multiplied by 2·33, accord-

[1] F. W. Fletcher (North London Chemical Works, Holloway) has devised a "thermo-hydrometer" which allows the temperature of the liquid in which it is immersed being read with great facility. One side of the ivory scale enclosed in the stem indicates the specific gravity of the liquid, while the other shows the temperature; the stem of a delicate thermometer, the bulb of which is situated below that of the hydrometer, running up inside the hollow hydrometer stem. An approximate correction for temperature may be made by adding one degree to the observed specific gravity for each 8° F. above 60°.

ing to Haeser and Christison; by 2, according to Trapp; or by 2·2, according to Loebisch; the products will be the number of grammes or grains of solids contained in 1000 c.c. or fluid grains of the sample. By dividing the product by 1000, and multiplying by the volume in centimetres or fluid grains passed in twenty-four hours, the total weight of solids excreted by the kidneys in that time will be found.[1] The variation in the factors given by the authorities quoted shows that, for reasons above stated, the method is only roughly approximate.

Bouchardat has proposed a somewhat similar formula for ascertaining approximately the proportion of sugar contained in diabetic urine from the specific gravity of the liquid. The gravity above 1000 is multiplied by 2, and the product by the number of litres of urine passed in twenty-four hours. From the product 60 is deducted, this correction representing the influence of the solids other than sugar, when the remainder will represent the weight of sugar in grammes passed in twenty-four hours.[2]

When an accurate determination of the solid matters of urine is required, it is best effected by evaporating an exactly weighed or measured quantity in a flat-bottomed porcelain or platinum capsule on the water-bath, exposing the apparently dry residue for some hours in the water-oven, and weighing the residue with precautions to prevent re-absorption of water. 5 c.c., or 100 fluid grains, is a suitable volume of urine to use for the determination.

[1] Thus if the urine passed in twenty-four hours measure 1450 c.c., and have a specific gravity of 1032·5, the total solid matters excreted by the kidneys in that time will be, by Haeser and Christison's formula,

$$\frac{32{\cdot}5 \times 2{\cdot}33 \times 1450}{1000} = 101{\cdot}1 \text{ grammes.}$$

[2] Thus, taking the same urine as before:—$32{\cdot}5 \times 2 = 65{\cdot}0$; $65 \times 1{\cdot}45 = 94{\cdot}25$. $94{\cdot}25 - 60 = 34{\cdot}25$, which figure represents the weight in grammes of sugar excreted by the kidneys in twenty-four hours.

DIABETIC URINE.

Glycosuria, or the excretion of urine containing a kind of sugar known as glucose, is especially characteristic of the disease known as *Diabetes Mellitus*.[1] It is commonly, but by no means invari-

[1] "According to the writings of Celsus, Aretæus and Galen, the disease termed 'diabetes' (διά, 'through,' βαίνω, 'I go') seems to have been recognised in a general way by the ancients. The progressive emaciation characteristic of the malady was observed as being accompanied by inordinate thirst, voracious appetite, and excessive discharge of urine. It was not, however, until 1674 that the urine in certain cases was discovered to possess a sweet taste, and the honour of this discovery, on which followed the establishing of the distinction between *diabetes insipidus* and *glycosuric diabetes*, is due to Willis, an English physician. A hundred years subsequently, Dobson, of Liverpool, discovered that the blood as well as the urine contained sugar; and he inferred therefrom that this sugar was separated from the blood, and not formed by the kidney. In 1778, Cowley separated the sugar from the urine in a free state. In 1815, Chevreul pointed out that the sugar existing in the urine in cases of *diabetes mellitus* was different from cane sugar and closely resembled that of the grape; and in 1825 Tiedmann and Gmelin ascertained that during its passage along the alimentary canal starchy matter was transformed into sugar."—*Urine and Urinary Analysis*, by D. Campbell Black.

Glycosuria may be temporarily induced (so long as the liver is charged with glycogen) by puncturing the floor of the fourth ventricle of the brain, while the lesion of a closely adjacent part of the same ventricle has been found to produce polyuria. Hence it has been suggested that in cases of diabetes in which both glycosuria and polyuria are present both these localities of the fourth ventricle have suffered irritation or injury.

Complete removal of the pancreas from an animal produces a condition of diabetes, which is relieved if the pancreas from another animal be grafted into the abdomen of that from which the pancreas was extirpated. Similarly, disease of the pancreas in man causes diabetes.

Diabetes kills the person suffering from it by starving the tissues and emaciating the body. Sometimes death ensues from uremia due to exhaustion of the kidneys and their consequent failure to excrete urinary materials from the blood.

The physiology of diabetes is very imperfectly understood. The appearance of sugar in the urine depends upon some failure at points where the sugar is

ably, associated with *Polyuria*, or the passing of an excessive volume of urine. This may exist without the presence of glycosuria, in which case the disease is known as *Diabetes Insipidus*.[1] In typical cases of *diabetes mellitus* the volume of urine excreted is frequently from 100 to 130 oz. per diem, and sometimes reaches twice this volume. An excretion of 400 oz. per diem has been observed.

Typical diabetic urine is very pale, probably owing to the dilution of the urinary pigments, but in mild cases, unaccompanied by marked polyuria, the urine has often a very dark colour. The odour sometimes resembles that of hay, but in advanced cases of diabetes the excretion has often an odour of acetone or alcohol. The taste of diabetic urine is distinctly sweet from the presence of sugar.

The urine of diabetic persons has a tendency to froth on agitation. Its reaction is usually distinctly acid, even after meals or alkaline medicines have been taken.

The specific gravity of typical diabetic urine is very

absorbed and assimilated, or at some other point (*e.g.*, the muscular tissue) where sugar properly assimilated in the blood fails to be oxidised and made use of. Sugar injected directly into the blood is not oxidised in the muscle, but runs off through a healthy kidney and is found in the urine. The significance of sugar in the urine of those who eat redundantly of sugar and other carbohydrates, who take little muscular exertion, and whose persons are already laden with fat, is quite different from that which it has in the case of persons who are emaciated. Similarly, sugar in the urine of an overcharged system is very different in its significance from glucose in the urine of the same person when all sugar has been stopped, the bulk of the starch has been excluded from the diet, and a fair amount of daily walking or other gentle muscular exercise is taken.

[1] Thudichum mentions a case in which 5600 c.c. (=196 oz.) of urine containing 0·8 per cent. of sugar was excreted in twenty-four hours, while at a later period the volume fell to 4333 c.c., and contained only traces of sugar. In many cases of diabetes with a minimum excretion of sugar, no material increase in the volume of urine is observed, though the patients have a strong desire to micturate. In these cases it is assumed that the sugar exerts an irritating action on the bladder, and causes its frequent evacuation. In many cases polyuria appears to be the result of an irritation of the nerves and consequent congestion of the kidneys.

high, the usual range being from 1030 to 1040; but a density of 1065 was observed by Seegen in a urine containing 10 per cent. of sugar.[1] Bernard states the maximum density at 1074.

Albumin is sometimes present in the urine in chronic cases of diabetes, but tube-casts are of rare occurrence.

Unless previously concentrated, diabetic urine does not usually deposit uric acid when acidified and left at rest. Whether this behaviour is due to the dilute condition of the excretion, or to the presence of less than the usual amount of uric acid, is not certain.

As a rule, diabetic persons excrete more urea than persons in health, owing to the greater amount of proteids they consume. There is no relation between the urea and the sugar excreted. The latter varies with the amount of starchy and saccharine food taken, except in cases where sugar is produced under a strictly albuminous diet.

Dieting experiments on diabetic patients show that more proteids are used than by healthy persons, since the carbohydrates are not available as a proteid-saving food. More fat undergoes combustion in the system of diabetics than in that of normal persons. Muscular work is stated to increase the excretion of urea, but not to affect sensibly the elimination of sugar.

The quantity of sugar excreted by diabetic persons varies from mere traces to as much as 600 grammes (20 oz.) in the twenty-four hours. The percentage of sugar present is, of course, dependent on the volume of the urine. In severe cases it not unfrequently reaches 8 or 10 per cent., and, according to A. H. Hassall, as much as 15 per cent. is present in some

[1] As a 10 per cent. solution of pure sugar in water has a specific gravity of 1040, the difference between this figure and the observed density may be regarded as due to the normal constituents of urine. Thus, eliminating the effect of the sugar, the urine would have had a specific gravity of 1025.

cases. The morning urine contains least sugar, while that passed three or four hours after a meal is the richest in sugar. Under strictly animal diet, the sugar will often fall to 1 per cent., while a meal of starchy or saccharine food will raise it to five or even to ten times that amount. Hence the pathological significance attaching to the excretion of 50 grammes of sugar daily when under strictly animal diet is much greater than the elimination of a larger amount when saccharine and amylaceous foods are being freely taken.

As the proportion of sugar in diabetic urine varies largely according to the time of day and the nature of the food taken, it is highly important that any analytical examination should be conducted on the united excretion of the previous twenty-four hours.

The question of the occurrence of traces of sugar in normal human urine has been the occasion of much controversy, and the last word on the subject still remains to be said. Brücke (*Wien. Akad. Sitzungber*, xxix. 346) appears to have been the first to state that all normal urine contained sugar, and this view was supported by Bence Jones (*Jour. Chem. Soc.*, xiv. 22), Kühne, Tuchen, and many other observers; but opposed by Friedlander, Wiederhold, Maly, and Külz. The question was re-examined in 1871, by Seegen, who pointed out many fallacies in the methods of those who had found sugar, and concluded that it was either absent from normal human urine, or present in such small proportion that the then existing methods were insufficient for its positive recognition in the presence of co-occurring substances which simulate many of its reactions. On the other hand, F. W. Pavy, in 1878, concluded that sugar was a normal constituent of urine, and that no sharp line of demarcation could be drawn between the

excretion in health and in diabetes, except quantitatively (*Guy's Hospital Reports*, xxi. 413). Molisch, from the examination of a large number of samples of healthy human urine by the alpha-naphthol and thymol tests, came to the conclusion that traces of sugar are met with frequently in human urine; but the value of his tests, and hence the accuracy of his conclusions, have been disputed by Leuken and also by Seegen (*Jour. Soc. Chem. Industry*, vi. 149, 150). E. Luther, again (*Chem. Centr.*, 1891, ii. 90, and *Jour. Chem. Soc.*, lx. 1559), as the result of the application of the furfuraldehyde and alpha-naphthol tests to a large number of samples, concluded that glucose is present in all human urine, the amount found in the excretion of adults averaging 0·1 per cent., while the total carbohydrates amount to 0·2 per cent. According to E. Roos (*Zeit. physiol. Chem.*, xv. 513), the normal urine of the dog, horse, and rabbit always contains more or less carbohydrates, as indicated by the furfuraldehyde reaction and confirmed by the benzoic chloride test. Human urine is stated by Roos always to give an affirmative reaction with phenyl-hydrazine, and the same is true of dogs' urine, while the excretion from rabbits gives especially well-formed crystals. The urine of all these animals was found to be slightly lævo-rotatory. Wedenski (*Zeit. physiol. Chem.*, xiii. 122), by agitating a large volume of urine with benzoic chloride, obtained a separation of the insoluble benzoyl compounds of carbohydrates. On separating the precipitate and treating it with soda, a portion dissolved and appeared to consist of animal gum, while the undissolved portion gave the reactions of dextrose.

G. Stillingfleet Johnson denies the presence of traces of sugar in normal urine, on the ground that strictly negative reactions were obtained on testing

the filtrate from the precipitate produced on treating the urine with mercuric acetate. But while mercuric acetate effectually and conveniently removes from solution the creatinine and other interfering substances, it also exerts an oxidising action on glucose itself, so that where mere traces are present they might be expected to suffer complete destruction. Direct experiments, made by the author to test the point, showed that the oxidation of the glucose was but trifling in the highly dilute solutions employed. The writer, by what was *apparently* substantially the same method as that by which G. S. Johnson obtained negative results, but confirmed by the results of the phenyl-hydrazine test,[1] satisfied himself that minute quantities of sugar are present in some specimens of urine from persons apparently in perfect health.

Similarly, relying on the failure of normal urine to yield a red-brown coloration with an alkaline solution of picric acid, after the creatinine, &c., have been removed with mercuric acetate, Sir G. Johnson maintains strongly the absence of sugar from the excretion; and meets the objection that such urine often gives a crystalline product with phenyl-hydrazine by the suggestion that, "for some reason, the test must be difficult to apply and uncertain in its results."[1] (See articles and correspondence in the *Lancet* during July and August, 1894.) It is contended that the mere presence of traces of sugar in urine thereby proves the excretion to be abnormal, though sugar admittedly appears temporarily on very slight provocation.[2] But such an argument begs the

[1] Glycuronic acid and its compounds give with phenyl-hydrazine a crystalline compound closely resembling phenyl-glucosazone, but having a different melting-point, but with proper precautions no confusion between the two bodies is possible. (See pages 41 and 88.)

[2] In the discussion of a paper by the author, "On the Examination of Urine for Small Quantities of Sugar," read before the Society of Public Analysts, Mr G. Stillingfleet Johnson is reported to have said:—"The question was,

very question at issue, which is whether normal urine does not sometimes contain traces of sugar.

The latest experimental contribution to the controversy is by F. W. Pavy (*Physiology of the Carbohydrates*, 1894), who, by operating on large quantities of urine, precipitating the sugar in combination with oxide of lead, and recovering it from this compound, obtained a liquid which exhibited the chief chemical reactions of glucose. No attempt appears to have been made to obtain the glucose in a solid form, and the method employed for effecting its fermentation by yeast is open to criticism.[1]

W. D. Halliburton (*Chemical Physiology and Pathology*, 1891) considers the balance of evidence clearly in favour of the existence of a small quantity of sugar in normal urine.[2]

What is a normal urine? This was simply reduced to the further question, Who is a healthy man? Everybody knew that a man might rise in the morning a healthy man, and that he might go to bed at night anything but a healthy man. He was quite sure that slight errors of diet—such, for instance, as taking a late dinner, or dining out—were sufficient to produce a temporary glycosuria, which was of no importance whatever. If the test were applied carefully to the urine of healthy individuals, it would be found in the long run to give practically negative results. He had no doubt that the reason why the idea that normal human urines were saccharine in character had existed so long was that the reducing action of creatinine had been mistaken for the reducing action of sugar."—*Analyst*, xix. 185.

[1] See a correspondence in the *Lancet*, January, February, and March, 1895.

[2] In a letter published in the *Lancet* for February 9th, 1895, Halliburton writes:—"I think that a careful study of the researches of Seegen and of Baisch, in which more stringent chemical methods were employed than in those of Dr Pavy, will convince the impartial observer that a small quantity of glucose is obtainable. Whether this is artificially produced by the method of analysis, as suggested by Sir George Johnson, is a subject that demands renewed research. Dr Pavy gives the percentage of sugar in normal urine about five times greater than has been found by these observers, and I think his high figure is most easily explicable by Sir George Johnson's hypothesis—namely, a neglect to take into consideration the reducing action of creatinine." This attempted explanation does not appear valid, since Pavy relies largely on the separation of the glucose in the form of an insoluble lead compound. It is nevertheless remarkable that not the slightest reference to creatinine or its proved reducing power on copper solutions, &c., is to be found throughout Dr Pavy's writings, and in a letter published in the *Lancet* for March 2nd, 1895, Pavy declines a direct challenge of Dr Halliburton to explain his views as to creatinine.

THE CARBOHYDRATES OF URINE.

The "sugars" belong to the class of organic compounds known to chemists as Carbohydrates. This name is a survival of the period when they were regarded as hydrates of carbon, or compounds of carbon with the elements of water, if not with water itself. Thus the carbohydrates contain carbon, hydrogen, and oxygen, the two latter elements being in the proportion in which they exist in water. Hence the carbohydrates, as a class, may be represented by the generic empirical formula:—$C_xH_{2n}O_n$. Great progress has been made of late years in the knowledge of the constitution of sugars and other carbohydrates, the researches of Emil Fischer being of pre-eminent importance. Fischer has shown that the class of sugars known as glucoses, of which grape-sugar is the type, are really the aldehydes or ketones of the hexatomic alcohol mannite or mannitol, having the formula:—$C_6H_8(OH)_6$. Just as aldehyde is obtained by oxidising ordinary alcohol, C_2H_6O, so from mannitol, $C_6H_{14}O_6$, the corresponding compound, $C_6H_{12}O_6$, is obtained. The carbohydrates of this composition are called *Monosaccharids*, the best known members of the class being dextrose (sometimes called dextro-glucose, or simply glucose), lævulose, and galactose. By the loss of the elements of water, two molecules of these glucoses may coalesce to form *Disaccharids*, thus:—$2C_6H_{12}O_6 - H_2O = C_{12}H_{22}O_{11}$. The disaccharides thus formed, when boiled with dilute acids, readily take up the elements of water again, becoming split into two molecules of monosaccharid. These two molecules may be either identical or dissimilar in nature. Thus, maltose splits into two molecules of dextrose; cane-sugar yields one of dextrose and one of lævulose; while milk-sugar is hydrolysed to dextrose and galac-

tose. *Trisaccharids*, resulting from the coalescence of three molecules of glucose, are known, the typical member of this class being raffinose, $C_{18}H_{32}O_{16}$.

Starch and dextrin are still more complex molecules, but, like the more simply constituted saccharides, tend to hydrolyse into their constituent disaccharide and monosaccharide molecules under the influence of dilute acids or other hydrolysing agents. The following represents the chief features of existing knowledge on the subject :—

Saccharid.	Hydrolysis Products.
α- and β-Amylan.	Dextrose.
Cellulose.	Dextrose.
Dextrin.	Dextrose.
Glycogen.	Dextrose.
Inulin.	Lævulose.
Lichenin.	Dextrose.
Tunicin.	Dextrose.
Starch.	Dextrose.
Maltose.	Dextrose.
Lactose (milk-sugar).	Dextrose and Galactose.
Cane-Sugar.	Dextrose and Lævulose.
Raffinose.	Dextrose, Lævulose, and Galactose.

Dextrose. Dextro-glucose. Glucose. Grape-sugar. $C_6H_{12}O_6$; $CH_2(OH).(CHOH)_4.COH$; or, $CH_2(OH).CH(OH).CH(OH).CH(OH).CH(OH).COH$.[1] Dextrose is a constant, or at least a very frequent, product of the hydrolysis of the polysaccharides by boiling with dilute acids. It is also produced by the hydrolysis of the natural glucosides amygdalin, populin, salicin, hesperidin, lupulin, phloridzin, and ruberythric acid. Æsculin, arbutin, and coniferin also yield dextro-rotatory glucoses on hydrolysis, but it is not certain that they are identical with

[1] This constitutional formula shows the presence of the COH group, and consequently classes dextrose among the aldehydes. On the other hand, lævulose contains a carboxyl group, CO, and has the constitution of a ketone :—$CH_2(OH).CH(OH).CH(OH).CH(OH).CO.CH_2(OH)$.

ordinary dextrose. On the other hand, lævulose is obtained when inulin is boiled with dilute acids. Dextrose occurs in the free state in grapes, many other fruits, the seeds and sap of plants, &c. It also occurs in the blood, liver, and other parts of the body, and is the characteristic sugar of diabetic urine.[1] Dextrose often crystallises from honey, in which it co-exists with lævulose. As already stated, a mixture of dextrose and lævulose in equal proportions results from the action of dilute acids on cane-sugar, and it is also formed by the hydrolysis of milk-sugar and maltose.

Dextrose usually crystallises from its aqueous solution in granular hemispherical warty masses or tabular crystals, containing $C_6H_{12}O_6 + H_2O$, but hot concentrated solutions often deposit anhydrous dextrose in prisms. Dextrose loses its water of crystallisation when gently heated. The anhydrous substance melts at 146°, and at about 170° C. loses water and is converted into dextrosan, $C_6H_{10}O_5$, and at higher temperatures (200° C.) yields caramel.

Dextrose is less soluble than cane-sugar in cold water, requiring $1\frac{1}{3}$ times its own weight, but it dissolves in all proportions in boiling water, forming a syrup having a sweetening power inferior to a solution of cane-sugar or lævulose of the same strength.

Solutions of dextrose exert a powerful dextro-rotatory action on polarised light, and one of the most accurate methods of estimating dextrose, in the absence of other optically active substances, is based on this fact. The specific rotation of dextrose solutions for a concentration of 10 per cent. is $+52{\cdot}7°$ for the sodium ray, and $+57{\cdot}0°$ for the transition-tint.

When treated with yeast and exposed to a moderate temperature, solutions of dextrose readily undergo the

[1] It is not absolutely certain that the dextro-rotatory glucose of diabetic urine is strictly identical with the sugar of grapes.

alcoholic fermentation, the weight of alcohol generated being approximately half that of the glucose fermented. The best conditions for obtaining quantitative results are described on page 46, *et seq.*

Dextrose is not affected when heated for a moderate time with dilute acids. Prolonged treatment is said to result in the formation of products having the probable formula $C_6H_{14}O_7$. When quite pure, dextrose is not readily charred by concentrated sulphuric acid, but combines with it to form a compound decomposed by water. By treatment with nitric acid, dextrose is oxidised to saccharic acid, $C_6H_{10}O_8$.

When a caustic alkali is added to a solution of dextrose, the liquid acquires a colour ranging from yellow to dark brown, according to the amount of glucose present. The change of colour occurs slowly in the cold, but almost instantaneously on heating. The reaction results in the formation of acetal, acetone, and formic, acetic, and lactic acids; besides the little-known bodies termed glucinic, japonic, and saccharumic acids. On acidulating the brownish liquid with nitric acid, the colour is lessened or destroyed, and the solution acquires the characteristic odour of caramel (burnt sugar).

Moore applied the foregoing facts to the detection of sugar in diabetic urine. The sample to be tested is first freed from albumen, if present, by rendering it faintly acid with acetic acid and boiling. The filtered liquid, or, in the absence of albumin, the original urine, is mixed with an equal measure of normal caustic soda or potash (or *Liquor potassæ*, B.P.), and filtered, without heating, from the precipitated earthy phosphates. The filtered liquid is then boiled in a test-tube, in such a manner that only the upper part is heated, when in presence of much glucose a yellow or brown colour will be developed in the heated part of

the solution, and contrasts well with the unchanged lower portion. The test is simple, and occasionally very useful, but does not indicate with certainty less than 2 per cent. of glucose. Cane-sugar, uric acid, and creatinine give no similar reaction. Urine containing the colouring matters of rhubarb and senna becomes reddish-brown with alkali before heating; while samples containing catechol (pyrocatechin) acquire a brown colour on exposure to air.

A solution of dextrose dissolves the alkaline earths, forming yellow solutions precipitated by alcohol. By boiling with excess of lime dextrose is rapidly acted on and destroyed.

Dextrose, when pure, is not precipitated by neutral or basic lead acetate, but on subsequent addition of ammonia it yields a white insoluble compound of dextrose and lead hydroxide, which may be washed without decomposition if carbonic acid be excluded. This lead compound affords a means of isolating dextrose from urine and freeing it from various co-occurring substances. (See page 43.)

When heated with water and silver oxide, dextrose yields glycollic, oxalic, and carbonic acids, but not acetic acid.

Dextrose is a powerful reducing agent, especially in presence of free alkali and at a high temperature. Thus at the boiling-point, in alkaline solution it reduces silver, mercury and bismuth to the metallic state, cupric oxide to cuprous, ferricyanides to ferrocyanides, blue indigo to white indigo, &c., &c. Further details of these and similar reactions, and their application to the detection of urinary sugar, are given on page 50, *et seq.*

By the action of nascent hydrogen in neutral or alkaline solution, but not in presence of acid, dextrose is reduced to mannitol, $C_6H_{14}O_6$.

With phenyl-hydrazine dextrose reacts to form a well defined osazone, crystallising in tufts of needles. The properties and method of preparing this important body are fully described on page 86, *et seq.*

On agitating a solution of dextrose with benzoyl chloride, a compound of the two separates in white crystals. This reaction has been employed to demonstrate the presence of glucose in urine.

LÆVULOSE. LÆVO-GLUCOSE. FRUIT SUGAR. $C_6H_{12}O_6$; or $CH_2(OH).CH(OH).CH(OH).CH(OH).CO.CH_2(OH)$. Lævulose occurs with dextrose in honey and in many fruits, and is a product of the hydrolysis of cane-sugar and raffinose. The hydrolysis of the starch-like body inulin yields lævulose without dextrose. The conversion of dextrose into lævulose has been effected by E. Fischer by acting on the former with phenyl-hydrazine, reducing the resultant glucosazone to isoglucosamine by zinc and acetic acid, and treating the last body with nitrous acid.

Lævulose is separated from dextrose by converting them into the lime compounds, when the former sugar forms an insoluble product, which may be separated from the soluble lime dextrosate, washed, and decomposed by oxalic acid.

Lævulose is generally described as a syrup, but on treating this with absolute alcohol the lævulose separates after some time as a mass of fine shining needles, which melt at 95° and undergo partial decomposition at 100° C.

With phenyl-hydrazine in excess, lævulose reacts to form an osazone said to be identical with that yielded by dextrose under similar treatment.

In its other chemical reactions lævulose presents the closest resemblance to dextrose, but differs from it in the characters of its lime compound as already mentioned, and in being readily affected by treatment

with dilute acids, or even by boiling its aqueous solution. Formic, propionic, and other acids are formed by boiling lævulose with dilute hydrochloric or sulphuric acid.

Lævulose also differs from dextrose in the product of the action of bromine. When a solution of dextrose is heated with bromine-water, and the liquid then treated with silver oxide (care being taken to avoid an excess of the latter), gluconic acid, $HC_6H_{11}O_7$, is formed, and may be obtained as a syrup on evaporation. If slaked lime be added in excess to its lukewarm solution, and the liquid filtered and heated to boiling, the acid is almost completely precipitated as a basic calcium gluconate. When lævulose is similarly treated with bromine-water and oxide of silver, it yields glycollic acid, $HC_2H_3O_3$, the calcium salt of which crystallises in silky needles, which require about 90 parts of cold water or 18 of boiling water for solution.

The most characteristic distinction between dextrose and lævulose is their action on polarised light. Thus, while dextrose, as its name denotes, exerts a dextrorotatory action, lævulose is still more strongly lævorotatory; and while the optical activity of dextrose is unaffected by temperature the activity of lævulose rapidly diminishes as the temperature increases. Thus at about 87° C. its specific rotation for the D ray is $-52{\cdot}7°$, being exactly the same as that of dextrose, but in the opposite direction. At 20° C., Hönig and Jesser give the specific rotation as $-90{\cdot}72°$ for a 10 per cent. solution. Jungfleisch and Grimbert (*Compt. rend.*, cvii. 390) give the following formula for the specific rotation of solutions of lævulose for temperatures (t) between 0° and 40° C. and concentrations (p) less than 40 per cent.:—

$$[\alpha]_D = -101{\cdot}38 - 0{\cdot}56t + 0{\cdot}108\ (p - 10).$$

The rotation decreases by 0·56 degrees for every rise of 1° C. in temperature. In a later paper (*Compt. rend.*, cviii. 144) they find that the rotation of lævulose prepared from inulin is −90·02°, while that obtained from invert-sugar exhibits a rotation of −95·74°; both at a temperature of 20° C. This they attribute to the influence of the mineral acid used for inverting the cane-sugar, the same difference not being observed when acetic acid was substituted, but becoming at once apparent on adding hydrochloric acid. Ost (*Berichte*, xxiv. 1636) has to some extent confirmed the foregoing observations, and gives the following formula for the specific rotation of pure lævulose at 20° C. p is the weight in grammes of lævulose in 100 grammes of the solution:—

$$[\alpha]_D^{20^\circ} = -(91{\cdot}90^\circ + 0{\cdot}111p).$$

Lævulose is stated by some observers to occur at times in the urine of diabetic persons. Its presence has been commonly inferred from discordance between the estimations of glucose by the polarimetric and oxidation methods. But as lævulose does not appear ever to have been actually isolated from urine, the evidence of its presence is at best inconclusive. On *a priori* grounds, the existence of lævulose in urine under ordinary conditions is improbable, as it is much more oxidisable than dextrose. This fact is so well-established that the use of lævulose is actually permitted to diabetic patients.[1] According to J. B. Haycraft, patients suffering from chronic diabetes can oxidise 50 grammes (= $1\frac{3}{4}$ oz.) of lævulose per diem. In some acute cases, a portion of the lævulose is burnt off, a second converted into and

[1] Lævulose is now an article of commerce, and can be obtained at a price of 3s. 6d. per lb. from Messrs A. & M. Zimmermann, 6 and 7 Cross Lane, London, E.C.

excreted as dextrose, while a third passes into the urine unchanged. The same result may be expected from the ingestion of cane-sugar. In rabbits, lævulose undergoes conversion into glycogen, which accumulates in the liver.

H. Leo (*Chem. Centr.*, 1887, page 193) isolated from diabetic urine a reducing substance which, after being purified from the co-occurring dextrose by repeated treatment with a solution of barium hydroxide in methyl alcohol, was obtained as a bright yellow syrup. It was lævo-rotatory ($\alpha_D = -36{\cdot}07°$), was not fermentable with yeast even after boiling with hydrochloric acid, and had a cupric oxide reducing power much less than that of dextrose. The body was a carbohydrate of the formula $C_6H_{12}O_6$.

MILK-SUGAR or LACTOSE, $C_{12}H_{22}O_{11},H_2O$, has been found in the urine of nursing women by Biot, Kaltenbach, Sinetz, and others. It was isolated by Hofmeister (*Zeit. physiol. Chem.*, i. 101) by precipitating the liquid with neutral lead acetate, treating the filtrate with ammonia, and precipitating the refiltered liquid once again with lead acetate and ammonia. These two last precipitates were decomposed by sulphuretted hydrogen, the filtrate shaken with oxide of silver, and the filtered liquid freed from silver by sulphuretted hydrogen. Barium carbonate was next added, and the liquid evaporated. On treating the residue with alcohol, the milk-sugar was dissolved, and obtained in a crystalline form by evaporating the solution *in vacuo* over sulphuric acid. (See fig. 1.) Kaltenbach obtained mucic acid and galactose from the body thus isolated, thereby conclusively identifying it with milk-sugar.

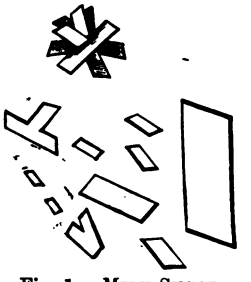


Fig. 1.—MILK-SUGAR.

Milk-sugar yields with the phenyl-hydrazine test a crystalline phenyl-lactosazone, somewhat resembling the analogous compound given by dextrose; but the crystals are much broader than those of phenyl-glucosazone, melt at 200° C. instead of at 204°, and dissolve more readily in alcohol.

Milk-sugar reduces alkaline solutions of copper, and has a specific rotation of +55·8° for the sodium ray.

When boiled with dilute mineral acids (citric acid has no action) milk-sugar is split up into two glucoses, dextrose and galactose. The latter has been identified with the sugar obtained by Thudichum by the action of dilute sulphuric acid on certain constituents of the brain, and called by him cerebrose.

Researches by F. W. Pavy (*Physiology of the Carbohydrates*, 1894) also appear to show that a reducing sugar analogous to, if not identical with, galactose is produced by the direct treatment of proteid matters with dilute sulphuric acid. After this treatment the product is nearly neutralised with baryta, and the neutralisation completed with barium carbonate. The filtered liquid is concentrated, and heated on the water-bath for at least an hour with acetic acid and phenyl-hydrazine (or phenyl-hydrazine hydrochloride and sodium acetate). Crystals of an osazone occasionally make their appearance in the hot liquid, but more frequently are deposited during and after cooling. From the crystalline osazone thus obtained the sugar itself is susceptible of recovery by the process described by Fischer. The "sugar" thus obtained gives a clear and definite reaction with Fehling's solution, attended with distinct deposition of red cuprous oxide; and it yields a crystalline osazone melting at 189° to 190° C.[1] It is, however, optically inactive, and unfermentable with yeast.

[1] Galactosazone melts at 193° to 195° C.

MALTOSE, $C_{12}H_{22}O_{11}$, is said to have been found in diabetic urine by Le Nobel. It is dextro-rotatory ($\alpha_D = +138{\cdot}9°$), reduces alkaline solutions of copper, &c., and yields with phenyl-hydrazine a compound which crystallises in yellow tables melting at 82° C.

SUCROSE or CANE-SUGAR, $C_{12}H_{22}O_{11}$, is alleged to be liable to occur in the urine of persons who consume large quantities of sugar. It is dextro-rotatory ($\alpha_D = +66{\cdot}5°$), does not reduce alkaline cupric solutions, and forms no compound with phenyl-hydrazine.

DEXTRIN, $C_6H_{10}O_5$. In some cases of diabetes in which the sugar gradually disappeared, Reichard (*Pharm. Zeitschrift für Russland*, xiv. 45) found Fehling's solution to become gradually green, then yellow, and sometimes finally dark brown. He suspected this to be due to the presence of *dextrin*, which he succeeded in isolating by the following process. The urine was evaporated to a syrup, absolute alcohol and caustic potash added, the precipitate allowed to settle, and the liquid decanted. The precipitate was washed with absolute alcohol, and then dissolved in acetic acid. On repeated addition of absolute alcohol to this solution the dextrin was deposited as a white substance, which after drying was tasteless, gave the dextrin reaction with Fehling's test, a brown coloration with iodine solution, and was readily converted into glucose by boiling with dilute sulphuric acid.

ANIMAL GUM is a carbohydrate obtained by Landwehr by the action of hydrochloric acid on mucin, and said to occur in small quantities in urine. It forms an opalescent solution in water, the resultant liquid giving a sticky precipitate with ferric chloride and cupric sulphate solutions. It does not reduce alkaline cupric solutions until after boiling with dilute sulphuric acid. Landwehr's body yields oxalic acid

on oxidation with nitric acid, and lævulic acid by treatment with hydrochloric acid. It does not ferment with yeast, is precipitated by alcohol, and gives no coloration with iodine.

GLYCOGEN, $(C_6H_{10}O_5)_n$, has been termed "animal starch," and presents the closest analogy to soluble starch. It was first found in the liver, but has more recently been met with in many other parts of the body.

Pure glycogen is a white, amorphous powder, readily soluble in water to form a solution which is usually, but not invariably, opalescent, and becomes more limpid on adding acetic acid or an alkali. Glycogen is precipitated from its aqueous solution by alcohol whenever the alcohol amounts to 60 per cent. of the liquid. If the solution be quite free from salts, the separation is sometimes very difficult, but takes place instantly on adding a minute quantity of common salt. The precipitation of glycogen in liquids containing 60 per cent. of alcohol distinguishes it from the different varieties of dextrin, none of which are precipitated by alcohol of less than 85 per cent. strength. Glycogen exactly simulates dextrin in its behaviour with a solution of iodine, which produces a port-wine colour, disappearing on heating, and returning as the liquid cools.

Glycogen is strongly dextro-rotatory, the value of $[\alpha]_D$ varying from $+203^\circ$ to $+234^\circ$, according to the concentration of the solution.

Glycogen does not reduce Fehling's solution. It is precipitated by baryta-water as $BaO(C_6H_{10}O_6)_3$, and by basic lead acetate as $PbO(C_6H_{10}O_6)_2$.

When boiled with dilute nitric acid, glycogen yields oxalic acid. Boiled with dilute sulphuric or hydrochloric acid, it is converted into dextrose. It does not ferment with yeast, but diastase and saliva convert it

into maltose and achroo-dextrin, a little dextrose being also formed. On the other hand, in the hydrolysis of glycogen in the liver, dextrose and not maltose is the chief product.

Glycogen is best prepared by rapidly cutting up the liver of an animal killed immediately previously, and throwing the fragments into five times their weight of boiling water. After boiling for a short time, the fragments of liver are mixed with sand and reduced to powder in a mortar, and then returned to the water, which is again boiled. The liquid is strained, and faintly acidified with acetic acid while still hot. The filtrate from the coagulated proteids is rapidly cooled, and the remaining proteids precipitated by the alternate addition of hydrochloric acid and potassio-mercuric iodide. The filtered liquid is mixed with such a volume of strong spirit as to make it contain 60 per cent. of absolute alcohol, when the precipitated glycogen is filtered off, washed first with 60 per cent. spirit, and then with absolute alcohol and ether.[1]

The researches of Claude Bernard first established the fact of the formation of a substance in the liver which, from the circumstance of its ready conversion into glucose, he termed "glycogen." Bernard

[1] Brautigam and Edelmann (*Chem. Centr.*, 1894, i. 485; *Jour. Chem. Soc.*, lxvi. ii. 336) have proposed to distinguish horse-flesh from the flesh of other animals by a reaction based on its high content of glycogen. Ten per cent. of horse-flesh or liver can be detected by this means, the proportion present ranging from 0·37 to 1·07 per cent., while the flesh of other animals used for food contains little or none—ox-flesh coming next with 0·20 per cent. The finely-divided flesh is boiled with four times its weight of water, and the resulting broth treated with dilute nitric acid to precipitate albumin, and filtered. Saturated hydriodic acid is now added, so the two liquids may remain as distinct layers, when a red or violet zone will be seen at the junction of the two strata if glycogen be present. The reaction is said to be quite characteristic of horse-flesh. In the event of the extraction of the glycogen with water proving inadequate, a solution of caustic potash containing KOH equal to 3 per cent. of the weight of the flesh must be substituted. Humbert substitutes a saturated solution of iodine in boiling water for the hydriodic acid (*Jour. Pharm. et Chim.*, 1895, 195; *Analyst*, xx. 95).

affirmed, as the result of his experiments, that glycogen is normally, and as a physiological action of the liver regularly, transformed into glucose; that in health, this glucose, after being carried into the circulation by the hepatic veins, is destroyed by oxidation; but that in certain abnormal conditions this natural transformation is arrested. These views of Bernard have been strongly combated by Pavy and others, according to whom sugar is not found in the liver when examined instantly after death, and is not present in any quantity in the blood of the right side of the heart, as it would be if sugar were constantly being formed in the liver and conveyed away from that organ by the hepatic vein.[1]

Researches by Voit, Otto, Abbott, Lusk, and others render it probable that the formation of glycogen in the liver may arise in two ways: as a temporary store of carbohydrate food, and as the result of proteid metabolism. Doses of dextrose, lævulose, cane-sugar, and maltose increased the hepatic glycogen, while lactose and galactose did not, or only very slightly, but both these sugars increased the proportion of sugar eliminated in diabetes. In the alimentary canal, cane-sugar and maltose undergo inversion. Lævulose, lactose, and galactose are absorbed as such, and after large doses pass unchanged into the urine. The liver appears unable to form glycogen from sugars which, like lactose and galactose, do not pass into the condition of either dextrose or lævulose. Dextrose and lævulose appear to be the only kinds of sugar which, when present in the blood, lead to the storage of glycogen in the liver, and the liver-cells

[1] The blood in the right side of the heart consists of the venous blood returned from the whole body, *plus* that returned from the liver by the hepatic vein. The amount of sugar in the blood of the hepatic vein is the datum requisite for completing the argument.

either convert lævulose into glycogen direct, or after previously changing it to dextrose.

If carbohydrates be introduced into the stomach or blood of rabbits, the liver of which has been rendered quite free from glycogen by six days' starvation, a large amount of glycogen will be found in the liver after a few hours.

It is probable that the glycogen stored in the liver and the muscles is not derived exclusively from the carbohydrates of the food. It appears that the albuminous and gelatinous constituents of the food are also concerned in its formation. Thus animals which have been fed wholly on lean meat for a considerable period exhibit large stores of glycogen in their liver and muscles. In the severer forms of diabetes, under a protracted and exclusive flesh diet, the secretion of sugar does not cease, and even increases in proportion to the amount of albumin consumed.

Von Mering found that when phloridzin, a glucoside contained in the root-bark of cherry and apple trees, was administered to a dog, sugar appeared in the urine after a few hours. This glycosuria ceased in a few days, and the liver and muscles are then wholly free from glycogen. On again administering phloridzin, a still larger amount of sugar was excreted. Unless the sugar was produced from proteids, it must be assumed to have been formed from fat, for which assumption there is no foundation.

INOSITE, $C_6H_{12}O_6$, sometimes improperly called "muscle-sugar," has been met with in healthy urine after the use of diuretics or a large excess of water. Dahnhardt isolated 0·1 gramme of inosite from 8 litres of ox urine.

Inosite was found by Gallois in the urine of five diabetic persons out of a total of thirty examined.

Five of these samples contained sugar in various proportions in addition to inosite. Inosite has also been found in the urine in cases of albuminuria, syphilis, phthisis, and typhus fever.

For the detection of inosite, several litres of the urine should be treated with excess of neutral lead acetate, filtered, and the filtrate warmed and treated with basic lead acetate till precipitation is complete. The liquid is filtered after standing forty-eight hours, and the washed precipitate suspended in water and decomposed with sulphuretted hydrogen. The filtrate deposits uric acid on standing, when it is again filtered, evaporated to a syrup at 100°, and treated with absolute alcohol. The precipitate is dissolved in hot water, and three or four volumes of rectified spirit added. Ether is then gradually added until a permanent turbidity is produced, when the inosite crystallises on standing.

Pure inosite crystallises in rhombic prisms sometimes grouped in rosettes, or, from its solution in hot rectified spirit, in shining scales, or in tables which, under the microscope, somewhat resemble cholesterin (fig. 2). The crystals easily effloresce, and sometimes turn red. When impure, inosite is often obtained in mammillated forms. It dissolves in 16 parts of cold water, and is soluble in rectified spirit, especially when hot, but is insoluble in absolute alcohol or ether. Inosite melts at 210° C., has a sweet taste, is optically inactive, and does not ferment with yeast, but by treatment with putrid albumen it is said to

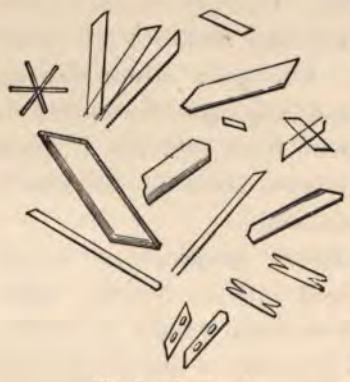


Fig. 2.—Inosite.

yield sarcolactic acid. Inosite does not reduce Fehling's solution. It is not precipitated by neutral lead acetate, but is precipitated by the basic acetate, especially on heating, as the compound $C_6H_{12}O_6,2PbO$. It yields no osazone with phenyl-hydrazine.[1]

According to Gallois, if an aqueous solution of inosite be treated in porcelain with a drop of solution of mercuric nitrate a yellowish precipitate will form, which, if spread out on the edge of the dish quickly and heated carefully, becomes dark red. On cooling, the colour disappears, but is reproduced on again heating. The reaction is not produced by glycogen, starch, mannite, milk-sugar, urea, uric acid, glycocine, taurin, or cystin. Albumin assumes a rose colour, and sugar becomes black under similar treatment.

According to Scherer (*Ann. Chem. und Pharm.*, 1852, page 375), if a solution of inosite be evaporated nearly to dryness at 100° with a few drops of nitric acid, and the nearly dry product moistened with ammonia and calcium chloride solution, a bright pink or rose-red coloration is obtained on completing the evaporation.

Glycuronic Acid. $C_6H_{10}O_7$; or $COH(CH.OH)_4.COOH$. Glycuronic acid doubtless has its origin in the dextrose of the body, to which compound it is closely related.[2] It was first obtained in the conjugated form of campho-glycuronic acid in the

[1] Although having the empirical formula of a glocuse, inosite is not a true carbohydrate. It really belongs to the aromatic series, and may be represented thus:—$CH(OH)\begin{cases} CH(OH).CH(OH) \\ CH(OH).CH(OH) \end{cases}CH(OH)$.

[2] The relation between glycuronic acid and bodies of the sugar group is shown by the following constitutional formulæ:—

Dextrose, ...	$CH_2(OH).(CH.OH)_4.CO.H$
Gluconic acid, ...	$CH_2(OH).(CH.OH)_4.CO.OH$
Saccharic acid, . . .	$CO(OH).(CH.OH)_4.CO.OH$
Glycuronic acid, . . .	$CO(OH).(CH.OH)_4.CO.H$
Gulonic acid, . . .	$CO(OH).(CH.OH)_4.CH_2.OH$
Gulose, ...	$CO(H).(CH.OH)_4.CH_2.OH$

urine of dogs to which camphor had been administered, and subsequently as uro-chloralic acid after the administration of chloral. It is remarkable for its tendency to form ethereal or glucosidal compounds when appropriate substances are introduced into the body. Traces of such compounds probably occur normally in urine, especially indoxyl- and skatoxyl-glycuronic acids; in addition to the combination with urea, having probably the constitution of uro-glycuronic acid, which appears to be the ordinary form in which glycuronic acid exists in urine.

Baeyer (*Annalen*, clv. 257) has shown that euxanthic acid, which exists in combination with magnesia in the "purrée" or "Indian yellow" of commerce,[1] is decomposed on boiling with hydro-

[1] *Piuri* or *Purrée*, now used as a pigment under the name of "Indian yellow," is obtained in Bengal from the urine of cows which are fed exclusively on the leaves of the mango tree and water. The urine is heated, and the precipitate separated and dried. Analyses of very pure specimens of purrée by C. Graebe (*Annalen*, cclv. 265) showed: euxanthic acid, 51; silica and alumina, 1·5; magnesia, 4·2; lime, 3·4; and water and volatile substances, 39 per cent. The analyses of Stenhouse and Erdmann show much less lime. Urea, uric acid, and hippuric acid have also been found in purrée. The poorer qualities contain considerable quantities of euxanthone, partly free and partly in combination. For the isolation of the euxanthic acid and euxanthone, and the assay of purrée, the colouring matter should be triturated with dilute hydrochloric acid until the whole has assumed the bright yellow colour of euxanthic acid. The residue is then well washed with cold water to remove the salts, and the euxanthic acid extracted from the residue by ammonium carbonate solution. It is precipitated from the filtrate by hydrochloric acid, and purified by crystallisation from alcohol. The euxanthone, left undissolved by the ammonium carbonate, is treated with caustic soda, the solution precipitated with an acid, and the precipitated euxanthone shaken out with ether or filtered off and dried at 100°.

Euxanthic acid has the constitution:—

$$OH.C_6H_3\left\{\begin{matrix}O\\CO\end{matrix}\right\}C_6H_3.O.CH(OH).(CH.OH)_4.COOH.$$

It forms pale yellow needles, which melt at 156–158°. It has a sweet taste and bitter after-taste, is but slightly soluble in cold water, very sparingly in ether, but readily in boiling alcohol. Alkalies colour the solution deep yellow. Euxanthic acid does not reduce Fehling's solution, nor form a compound with phenyl-hydrazine.

Euxanthone is a neutral substance, crystallising in pale yellow needles, soluble in alkalies but not in dilute acids. It forms no compound with phenyl-hydrazine.

chloric acid or dilute sulphuric acid, with formation of euxanthone and an acid which has been shown by Spiegel (*Ber.*, xv. 1965) to be identical with glycuronic acid, $C_{19}H_{18}O_{11} = C_{13}H_8O_4 + C_6H_{10}O_7$. In fact purrée is the best material for the preparation of glycuronic acid, which can be obtained on the small scale by the following process:—The artists' water-colour known as "Indian yellow" is ground up with sand, and then treated with dilute hydrochloric acid, which dissolves out calcium and magnesium salts, &c. The residue is washed with water and treated with a solution of ammonium carbonate, which dissolves the euxanthic acid, leaving euxanthone and sand undissolved. From the filtered liquid the euxanthic acid is precipitated by dilute hydrochloric acid, washed with cold water, and then heated with water in a closed soda-water bottle to 125° C. for three or four hours. The requisite temperature can be conveniently obtained by immersing the bottle in a bath of molten paraffin wax (candles). From the cooled product the euxanthone is dissolved by agitation with ether, and the glycuronic anhydride crystallised from the concentrated aqueous liquid.

Glycuronic acid is a syrupy liquid, miscible with water or alcohol. When the aqueous solution is boiled, evaporated, or even allowed to stand at the ordinary temperature, the acid loses the elements of water and yields the anhydride or lactone.

Glycuronic Anhydride, $C_6H_8O_6$, forms monoclinic tables or needles, having a sweet taste, and melting at about 160° when heat is gradually applied, or at 170–180° when heated rapidly. The anhydride is insoluble in alcohol, but dissolves readily in water to form a dextro-rotatory solution. $[\alpha_D] = 19{\cdot}25°$. The solution prevents the precipitation of cupric solutions by alkalies, and powerfully reduces hot Fehling's solution,

the cupric oxide reducing power being 98·8, compared with glucose as 100. On distillation with hydrochloric acid, glycuronic anhydride (as also uro-chloralic acid) yields furfuraldehyde, a trace of which substance is also obtainable on similarly treating normal urine.

Glycuronic acid itself is dextro-rotatory ($[\alpha]_D = +35°$), but many of its compounds are lævo-rotatory.[1] It reduces Fehling's solution on heating, and precipitates the metals from hot alkaline solutions of silver, mercury, and bismuth.

On oxidation, glycuronic acid yields camphoric and formic acids. By treatment with bromine it yields saccharic acid, $C_6H_{10}O_8$, a reaction which indicates the presence of an aldehyde group and the close relation between glycuronic acid and dextrose. Saccharic acid can again be reduced to glycuronic acid by treatment with sodium amalgam, further treatment yielding gulonic acid, $C_6H_{12}O_7$, a body which does not reduce Fehling's solution. (Fischer and Piloty, *Ber.*, xxiv. 521; *Jour. Chem. Soc.*, lx. 667.)

When boiled with caustic alkali, glycuronic acid yields oxalic acid as an invariable product. Catechol and protocatechuic acid are also formed if concentrated alkali be employed for the treatment.

Glycuronic acid is distinguished from glucose by not undergoing the alcoholic fermentation when treated with yeast. On the other hand, when fermented in presence of cheese and chalk it yields lactic and acetic acids.

Glycuronic acid forms a potassium salt which crystallises in needles. The sodium salt is similar. The zinc, cadmium, copper, silver, and calcium salts are uncrystallisable. The barium salt is amorphous

[1] After taking chloral hydrate the urine contains trichlorethyl-glycuronic acid ("uro-chloralic acid"), a lævo-rotatory body which is decomposed into trichlorethyl alcohol and dextro-rotatory glycuronic acid.

and soluble in water. It is the compound employed for the isolation of glycuronic acid from urine.

With phenyl-hydrazine, glycuronic acid forms a yellow crystalline compound melting at 114° to 115° C., but under modified conditions an amorphous, brownish-yellow body, melting at 150° C., is produced. According to J. A. Hirschl, normal urine yields this compound (see page 88).

For the actual isolation of glycuronic acid from urine a large quantity of the excretion is required. The method is described in an interesting report by H. H. Ashdown (*Brit. Med. Jour.*, 1890, i. 169, and *Pharm. Jour.*, [3], xx. 607).

Glycuronic acid occurs in the urine to a very notable extent after the administration of morphine, chloroform, chloral, butyl-chloral, nitrobenzene, camphor, curare, and certain other drugs. It was undoubtedly mistaken for glucose by the older observers. In one case recorded by Ashdown large amounts of glycuronic acid occurred in the urine of a healthy young man, whose excretion was not abnormal either in volume or density.

Glycosuric Acid is a body extracted by J. Marshall from pathological urine (*Arch. Pharm.*, [3], xxv. 593, and *Jour. Chem. Soc.*, lii. 1047). It is isolated by a process based on the insolubility of the lead salt in alcohol of 45 per cent. Glycosuric acid crystallises in opaque tetragonal prisms, which melt at 140°, are readily soluble in water, alcohol, and ether, less readily in chloroform, and insoluble in benzene, toluene, and petroleum spirit. Glycosuric acid contains no nitrogen, is readily and completely absorbed by animal charcoal, and appears to be a phenolic derivative. It reduces Fehling's solution more powerfully than glucose, and also reduces silver nitrate; but not bismuth compounds or alkaline solutions of picric acid.

It gives a brown coloration when boiled with aqueous soda. A dilute and neutral solution of ferric chloride gives an evanescent blue colour. On evaporating the ethereal solution of glycosuric acid at 60° the liquid takes a wine-red colour, which is imparted to the crystals which separate, but these form a colourless solution in water.

A. Geyger (*Pharm. Zeit.*, 1892, page 1488) extracted glycosuric acid from a diabetic urine by acidulating it with sulphuric acid and agitating with ether. The ether left on evaporation a crystalline substance, melting at 143° C., which proved to be the acid in question. He suggests that diabetic urine should always be examined in this manner.

DETECTION AND DETERMINATION OF SUGAR IN URINE.

A great variety of methods have been proposed for the recognition and estimation of sugar in urine. Many derive the only value they possess from being simple in application, and therefore not beyond the manipulative skill and appliances of the general medical practitioner. Not a few are utterly worthless and misleading in their indications, while others fail to show the presence of proportions of sugar (*e.g.*, below ½ per cent.) which are not without grave pathological significance, and of the greatest importance as a warning to the patient.

With the exception of a few colour-reactions of little value, the processes for detecting and determining sugar in urine may be arranged in the following classes :—

1. Isolation of the dextrose as such.
2. Methods based on the fermentation of the glucose.

3. Methods based on the optical activity of dextrose

4. Methods based on the reducing action of glucose. These include the various modifications of the copper, mercury, and bismuth tests, and the colour-changes produced by glucose in hot alkaline solutions of various coal-tar colours.

5. Methods dependent on the conversion of the sugar into definite crystalline compounds, such as those yielded with benzoyl chloride and phenyl-hydrazine.

Isolation of Sugar from the Urine.

When typical diabetic urine, containing from 5 to 10 per cent. of glucose, is in question, it is not difficult to isolate the dextrose in a pure state. On evaporating such urine on the water-bath to the consistence of a syrup, and then leaving it at rest for some days, dextrose will frequently separate in tabular crystals or irregular warty masses. More frequently it is deposited in crystals of a compound of dextrose with sodium chloride containing $2C_6H_{12}O_6,NaCl+H_2O$, which is less soluble in alcohol but more soluble in water than glucose itself. Not unfrequently the syrup may remain for months without any crystals appearing. Sometimes treatment of the syrup with ether, which is subsequently allowed to evaporate spontaneously, facilitates the formation of crystals. The syrup, with any crystals contained in it, is treated with cold absolute alcohol, which dissolves out urea and extractive matter, but leaves most of the glucose. On boiling the residue with rectified spirit the sugar is dissolved, sulphates, phosphates, urates, &c., remaining insoluble. The liquid is filtered hot and evaporated to a small bulk, when it will deposit crystals of glucose on cooling; these may be purified by recrystallisation from boiling rectified spirit.

For the isolation of the minute quantity of sugar existing in non-diabetic urine, F. W. Pavy employs the following adaptation of a process devised by Brücke:—A large quantity of the urine is treated with half its measure of a 10 per cent. solution of neutral lead acetate. The precipitate, which contains urates, sulphates, phosphates, colouring matter, &c., is filtered off, and the filtrate treated with basic lead acetate, any further precipitate being removed as before. The filtered liquid is then treated with ammonia and more lead acetate, unless a distinct excess has been already used. This causes the precipitation of the glucose as a definite compound with lead oxide. The precipitate is filtered off and washed with cold water till the greater part of the ammonia is removed, when it is suspended in water and decomposed by a stream of sulphuretted hydrogen. Sulphuric or oxalic acid may be substituted for the last reagent, but the product is more strongly coloured. In any case, the strongly acid liquid should be treated cautiously with sodium carbonate solution till neutral, when colouring matter will separate and may be filtered off. Further decolorisation may be effected by treating the liquid, rendered slightly acid with acetic acid, with a little animal charcoal (previously carefully purified from phosphates, &c., by boiling with hydrochloric acid and washing till free from acid reaction to litmus).

From this clarified liquid, dextrose can be obtained in crystals recognisable under the microscope, but for its identification by chemical tests the solution itself is quite suitable. Working on the product from healthy persons, Pavy obtained all the chief reactions of sugar, including the production of carbonic acid gas by fermentation with yeast and the formation of a crystalline compound with phenyl-hydrazine.

Pavy's results go far to decide in the affirmative the long-vexed question of the existence of traces of sugar in normal human urine (compare, however, page 20).

Fermentation Test for Sugar.

The recognition of sugar in urine, by its conversion into alcohol and carbon dioxide when treated with yeast, has the advantage of simplicity and almost absolute certainty that the effect produced is really due to sugar. On the other hand, the experiment extends over a comparatively long time, and the method is deficient in delicacy and accuracy.

In its simplest form, the fermentation test is applied by introducing a fragment of pressed yeast the size of a nut into a 4-oz. bottle, filling the bottle completely with the urine to be tested, and then inverting the vessel over a small capsule or saucer of the urine, in such a manner as not to allow any air to enter. The whole is then left in a warm place—from 25° to 30° C. (= 77° to 86° F.)—for twelve or fifteen hours, when, if sugar be present in quantity, the liquid will become lighter in colour, and diminish in gravity, while at the same time a notable quantity of carbon dioxide will collect in the upper part of the bottle. The test fails, or answers but imperfectly, when the proportion of sugar is less than 0·5 per cent. By keeping the bottle in an erect position, and allowing the fermentation to continue for twenty-four hours, a good approximation to the content of sugar may be obtained by noting the decrease in the density of the liquid. As pointed out by Sir W. Roberts, every degree of gravity lost by the fermentation corresponds closely with 1 grain of sugar per fluid ounce of the urine. Another portion of the same urine, untreated with yeast, should be kept side by side with the

fermentation test, and its density observed under similar conditions to the latter. Thus, if the untreated urine be found to have a gravity of 1042, and the portion treated with yeast have on completion of the fermentation a density of 1015, the urine will have contained 27 grains of sugar per fluid ounce (1042 − 1015 = 27). Attempts have been made to obtain more accurate results by this process, but the method has too many sources of error to make such refinements of practical value.

Instead of observing the diminution in density, the proportion of sugar may be deduced from the weight or measure of carbon dioxide (carbonic acid) gas evolved Many ways of effecting this have been devised, but one of the simplest and most effective is to introduce a known measure of the urine to be tested into a bottle furnished with a perforated cork, and attached by elastic tubing to the three-way tap of a nitrometer filled with brine (see "Urea"). Yeast is added to the urine, and the arrangement left for twenty-four hours in a warm place, as previously described. The gas which has collected in the nitrometer is then measured with the usual precautions. One c.c. of CO_2, measured over brine at the ordinary pressure and temperature (about 30 inches of barometric pressure, and at 15° to 20° C., = 59° to 69° F.), represents 0·002 gramme of glucose in the urine taken.

Instead of using a separate vessel for the urine, 5 c.c. may be introduced, through the tap, into a nitrometer filled with mercury, 5 c.c. of water containing as much yeast as possible in suspension run in through the tap, and the apparatus kept in a warm place until the fermentation is complete.

Where a nitrometer and the other appliances of a laboratory are not available, a very fair determination of the sugar in diabetic urine may be made by the

arrangement shown in fig. 3, constructed from a feeding-bottle and ordinary wash-bottle. The glass tube is removed from the inside of the feeding-bottle, and the india-rubber tube fixed air-tight into the stopper by means of sealing-wax. The nipple is removed from the other end of the india-rubber tube, which is then slipped on to the mouth-tube of an ordinary wash-bottle. Three fluid drachms (= ⅜ oz.) of the urine to be tested, or an equivalent measure of the diluted sample,[1] should then be placed in the feeding-bottle, about an ounce of water and a piece of pressed yeast added, and the stopper with its attached tubes firmly adjusted. The wash-bottle is filled with water, or preferably with brine, and its cork (carrying the tubes) firmly fixed in. This will cause the exit tube to fill with water. A suitable measuring vessel, such as a graduated 8-oz. medicine-bottle, is placed below the outlet tube so

Fig. 3.—Apparatus for estimating diabetic sugar by fermentation.

as to receive the brine displaced by the gas generated by the fermentation. The whole arrangement is kept at a temperature of from 25° to 30° C. (= 77° to 86° F.) for twenty-four hours, when the volume of

[1] 1 oz. of the sample may be diluted with 3 oz. of water, and 1½ oz. of the diluted liquid (= ⅜ oz. of the original sample) taken for the experiment.

liquid collected in the measuring vessel is observed as accurately as possible.

In any experiment where it is sought to determine sugar by fermentation, it is of course essential that the yeast employed be free from starch. Much of the pressed yeast met with in commerce contains a considerable proportion of starch, which is said to be added as a preservative, but is certainly not essential. The presence of starch may be readily detected by mixing up a little of the yeast with tincture of iodine, which will produce a blue or violet colour if starch be present.

If $\frac{3}{8}$ ounce (= 3 fluid drachms) was the measure of urine used for the experiment, every ounce of fluid displaced will represent 1 per cent. of sugar in the sample; while every 100 grains measure will correspond to 1 grain per fluid ounce of the urine.[1]

Fresh brewers' yeast may be substituted for pressed yeast if it be first well washed on a filter with cold water.

In all the foregoing modifications of the fermentation test it is important that the urine should be moderately but distinctly acid. The safest plan is to render the liquid slightly alkaline to litmus paper, to ensure the neutralisation of any possible mineral acid, and then to acidify the liquid with tartaric acid to the extent of about 0·5 gramme per 100 c.c. (2 grains per fluid ounce).

[1] The main reaction occurring in the fermentation of glucose is expressed by the equation $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. Hence 180 parts of sugar yield 92 of alcohol and 88 of carbon dioxide. (This statement is not strictly correct, but sufficiently so for the purpose in question.) 88 grammes measure 44,760 c.c. at the standard pressure and temperature, or about 48,000 c.c. at 20° C. (= 68° F.). Hence this measure of gas will be yielded by 80 grammes of glucose, 800 by 3 grammes, or 800 grains measure by 3 grains. If the urine contain 1 grain of glucose per fluid ounce, and $\frac{3}{8}$ oz. is employed, the liberated gas will measure $\frac{800}{3} \times \frac{3}{8} = 100$ grains measure. But if the content of sugar be 1 per cent., or 4·375 grains per fluid ounce, the gas will measure 437½ grains, or exactly 1 oz. If 15 c.c. of urine be taken, then every 10 c.c. of liquid displaced by the gas represents ¼ per cent. of sugar in the sample.

Polarimetric Determination of Diabetic Sugar.

The sugar contained in diabetic urine is commonly assumed to be identical with the dextro-glucose present in grapes and produced, together with lævulose, by the inversion of cane-sugar. It is not quite certain, however, that this assumption is correct, nor that the dextrose is not sometimes associated with lævulose or some other kind of sugar. The subject requires further investigation by competent observers, who will require to determine the total sugar by some more certain process than those commonly used for the purpose, comparing the results so obtained with those yielded by the optical method. In ordinary cases, however, the polarimeter can be trusted to give excellent comparative, if not rigidly accurate, results, provided the proportion of sugar is not too small. For the detection or determination of the traces of sugar supposed to be present in healthy urine, or even for the examination of slightly diabetic urine, the optical method is useless, since urine ordinarily contains traces of various substances other than sugar which are more or less optically active.[1]

The proportion of glucose in urine can be deduced from the following formula, in which c is the *concentration*, or grammes of sugar per 100 c.c. of urine

[1] M. Carles (*Jour. de Pharm.*, 1890, page 108) states that all normal urines have a lævo-rotation varying from 0·25 to 0·80° of the saccharometer, the precise activity varying with the proportion of extractive matter, intensity of colour and odour, amount of urea, and specific gravity. Carles inclines to the opinion that the optical activity of normal urine is due, at least in part, to the creatinine. He describes a urine which contained 33 grammes of urea and 2·27 of phosphoric acid per litre, and had a specific gravity of 1·025, which exhibited a left-handed rotation of 1·93°. Hence it might have contained 4·35 grammes of sugar per litre, and yet have been without dextro-rotation.

According to Haas, dextrose is the chief, and almost the only constituent of urine producing dextro-rotation, while lævo-rotation may be due to glycuronic acid, its phenyl and indoxyl derivatives, leucine, tyrosine, albumin, hemialbumoses, peptones, cystin, cholesterin, aspartic acid, pseudo-butyric acid, and lævulose. In addition may be mentioned the disturbing influence of quinine and other alkaloids.

a the *angular rotation* observed in the polarimeter; and l the *length* of the tube in decimetres,

$$c = \frac{a \times 100}{l \times 52{\cdot}7^{\circ}}.$$

This equation applies only to instruments in which monochromatic sodium light is employed. With saccharometers intended for observations of the transition-tint, 57·0° must be substituted for 52·7°.

In cases where the optical method may be suitably employed, its application is very simple.[1] Diabetic urine is usually so pale in colour that its rotation may be observed without previous clarification. If the colouring matter of the sample should occasion any difficulty in the observation, simple agitation of 100 c.c. of the cold urine with about 3 grammes of neutral acetate of lead, and filtration through paper, will generally produce an almost water-white filtrate.

Detection and Determination of Diabetic Sugar by its Reducing Action.

The methods of examining urine for glucose most generally employed are those based on its reducing action, which is powerfully exerted in boiling alkaline solutions. Thus the sugar of diabetic urine, which is chiefly if not entirely dextrose, *in hot alkaline solutions* effects the following reductions. The oxygen shown in the reactions is not evolved as such, but converts the glucose into gluconic acid and other oxidation-products.

(*a*) *Cupric oxide*, retained in solution by fixed organic matter, ammonia, or cyanides, is reduced to cuprous oxide :—$2CuO = Cu_2O + O$.

[1] The method of using the polarimeter is familiar to those accustomed to employ the instrument, and is described in great detail in various works readily accessible. A Laurent instrument on the half-shadow principle is to be preferred to other forms. A polarimeter of this kind, obtainable at a moderate price, is described in *The Analyst*, vol. xi. page 141. Another useful instrument is made by Messrs Philip Harris & Co., Birmingham.

(*b*) *Bismuthic oxide*, in the solid state, or in alkaline citrate solution, is reduced to metallic bismuth:— $Bi_2O_3 = Bi_2 + O_3$.

(*c*) *Mercuric oxide*, in the form of potassio-mercuric cyanide or iodide, and in presence of caustic alkali, is reduced to metallic mercury :— $HgO = Hg + O$.

(*d*) *Orthonitrophenyl-propiolic acid* is reduced to indigo-blue :— $2C_9H_5NO_4 = C_{16}H_{10}N_2O_2 + 2CO_2 + O_2$. *Indigo-blue* is, by further treatment, reduced to white indigo :— $C_{16}H_{10}N_2O_2 + H_2O = C_{16}H_{12}N_2O_2 + O$.

(*e*) *Ferricyanides*, in presence of fixed caustic alkali, are reduced to ferrocyanides :— $2K_3(C_6N_6Fe) + 2KHO = 2K_4(C_6N_6Fe) + H_2O + O$.

(*f*) *Picric acid* is reduced to picramic acid :— $C_6H_2(NO_2)_3.OH + 3H_2O = C_6H_2(NH_2)_3.OH \quad O_9$.

(*g*) *Methylene-blue* is converted into the corresponding leuco-compound :— $C_{16}H_{19}N_3SO = C_{16}H_{19}N_3SO$.

(*h*) *Safranine* is similarly reduced to the corresponding leuco-compound.

Some of the foregoing decompositions take place at the ordinary temperature, or considerably below the boiling-point, if time be allowed for the reaction.

The reaction which occurs when a solution of glucose is heated with an oxidising agent in presence of caustic alkali is complex, and has been but imperfectly studied. The principal products are said to be formic, oxalic, glycollic and carbonic acids, but the products of the action of the alkali itself on the sugar have evidently to be taken into account (compare page 24). The practical necessity of employing caustic alkali in conjunction with the oxidising agent is a grave defect of all quantitative tests based on the reducing action of glucose.

The recognition of glucose in urine, by its reducing action on the foregoing and similar yielders of oxygen, is readily effected when the proportion of reducing

sugar is considerable; but, when very small quantities are in question, the indications of the reagents are not to be depended on. This is not so much due to any deficiency in the delicacy of the reactions, as a consequence of the presence in urine of other bodies which resemble glucose in their power of exercising more or less reducing action on oxidising agents. Under abnormal conditions urine may contain many such reducing bodies, but even normal urine invariably contains traces of some of such bodies, and under special conditions of diet, or under the influence of medicines, these and others may be increased to a very material extent.

Of the constituents of normal urine which resemble sugar in their reducing action, the most important are uric acid and creatinine; but hippuric acid, creatine, and glycuronic acid and its compounds exercise a similar action, though their quantity is usually so insignificant that they may safely be neglected. Very little is known of the specific action of these urinary bodies on the before-mentioned oxidising reagents, and of their quantitative behaviour we know still less. The alkaline solutions of copper are almost the only reagents of which the oxidising power on urinary constituents other than glucose is even approximately known, and the published statements stand in much need of verification and revision. It may, however, be taken as an established fact that creatinine, uric acid, and glycuronic acid and its compounds reduce both Fehling's and Pavy's copper solutions. Creatinine also reduces an alkaline solution of picric acid, with production of a blood-red coloration, but uric acid does not reduce picric acid even on boiling. Safranine in alkaline solution is reduced to a leuco-compound by glucose, but not by creatine, creatinine, uric acid, or hippuric acid.

The following table shows the behaviour of various precipitants and alkaline oxidising agents when heated to boiling with the chief urinary constituents.

Reagent.	Albumin.	Glucose.	Uric Acid.	Creatinine.	Xanthine.	Remarks.
Neutral lead acetate.	Ppted.	Not affected.	Ppted.	Not affected.	Ppted.	...
Basic lead acetate.	Ppted.	Do.	Ppted.	Do.	Ppted.	...
Cupric acetate (acidulated).	Ppted.	Imperfectly reduced.	Ppted.	Partially ppted.	Ppted.	Glucose is gradually and imperfectly oxidised.
Mercuric acetate ($HgCl_2+2NaA$).	Ppted.	White ppte. of Hg_2Cl_2 gradually formed.	Ppted.	Ppted.	Ppted.	Creatinine ppted. very slowly in the cold. All others, except glucose, promptly.
Fehling's alkaline cupric tartrate solution.	Red-violet coloration.	Immediate separation of red Cu_2O.	Reduction and pptn. of cuprous urate.	Partial pptn. and reduction.	Reduction and pptn. as cuprous Xanthinate.	...
Pavy's ammoniacal cupric solution.	No change.	Colour discharged.	Colour discharged.	Colour discharged.	...	...
Gerrard's cyanocupric solution.	Do.	Do.	D	...	...	...
Nylander's bismuth citrate solution.	Do.	Pptn. of grey or black Bi.	Pptn. of Bi.	No change.	...	...
Knapp's mercuric cyanide solution.	...	Pptn. of grey Hg.	...	...	...	...
Sacchse's (Nessler's) mercuric iodide solution.	...	Do.	...	...	...	...
Braun's alkaline picric acid solution.	...	Brown-red colour.	No change.	Blood-red coloration.	...	...
Wender's alkaline methylene-blue solution.	...	Colour discharged.	Do.	Colour discharged.	...	...
Crismer's alkaline safranine solution.	...	Do.	Do.	No change.	...	...

When very small quantities of sugar are to be sought for, the removal of the interfering constituents of urine is absolutely necessary, and the neglect of this preliminary treatment has given rise to many erroneous observations on the presence of sugar.

Urine may be conveniently clarified, and freed from albumin (if present), uric acid, phosphates, sulphates, and colouring matters, by precipitation with neutral lead acetate. One-fourth of its measure of a 10 per cent. solution of lead acetate in water is sufficient to throw down all the precipitable matters from almost any urine. The precipitate is bulky, but filters readily, especially if the urine be first heated to boiling and the hot reagent added to the boiling liquid. This mode of operating avoids the risk of loss from bumping which is apt to occur when the liquid containing the dense lead precipitate is heated.[1] Addition of basic lead acetate to the filtrate from the precipitate produced by the neutral salt removes certain bodies which escaped precipitation previously, but there is no material advantage in its use. In either case, the filtrate from the lead treatment is colourless, or nearly so, and is well fitted for optical examination or for testing by the phenyl-hydrazine reaction.[2]

Mercuric chloride is a very perfect precipitant for urinary constituents. When sodium acetate is also

[1] Three per cent. of lead acetate in fine powder may be substituted for the solution prescribed in the text. The powder should be added to the boiling hot urine, which should then be well agitated and filtered. This plan avoids dilution and the consequent correction for the volume of reagent added.

[2] According to some observers, uric acid is incompletely precipitated by treating the urine with neutral lead acetate only, addition of basic acetate being requisite for its complete removal from solution. This is opposed to the author's experience, who has specially examined the filtrate from the precipitate by neutral lead acetate by the murexide test with negative results. It has been fully established that no sugar is carried down from solution by either neutral or basic lead acetate, but if ammonia be subsequently added glucose is completely removed as a compound with lead hydroxide, which can be washed without decomposition. This reaction is employed by Brücke and by Pavy for the isolation of sugar from urine.

added, as recommended by G. Stillingfleet Johnson, mercuric acetate is potentially present, and this is even a more perfect precipitant than the chloride. Johnson recommends that the urine should be treated with 5 per cent. of its volume of a cold saturated solution of sodium acetate, and 25 per cent. of a cold saturated mercuric chloride solution.[1] This treatment occasions an immediate voluminous precipitate containing albumin (if present), uric acid, xanthine, phosphates, colouring matters, &c. Creatinine is not precipitated immediately in the cold, but separates completely in the course of a few days, even at the ordinary temperature. If the liquid be boiled for a few minutes the creatinine is completely precipitated, and this behaviour affords by far the best means of separating it from urine. The urea is partially, but not completely, precipitated on boiling. Glucose is gradually oxidised at a boiling heat, but the reaction does not materially affect the detection of traces in the filtrate. The separation of the excess of mercury from the filtrate from the mercurial precipitate presents some difficulty. Sulphuretted hydrogen is not available for the purpose, as a fixed reducing body is stated by Johnson to be formed by its action, which body interferes with the subsequent detection of sugar. G. S. Johnson recommends the careful addition of ammonia, with subsequent filtration from the resultant precipitate. It is essential that the precipitation of the mercury should be perfect, as any remaining in the liquid will be reduced to metal by the glucose when the liquid is subsequently treated with caustic alkali and boiled, as it necessarily is whether Pavy's ammoniacal cupric solution or picric acid be employed for determining the glucose. The author has found

[1] The proportion of sodium acetate used by Johnson is considerably in excess of that requisite to react with the mercuric chloride.

ammonia uncertain in its action, and prefers to boil the liquid for five minutes or so with zinc-dust.[1] This precipitates the mercury perfectly, and the zinc, which passes into solution in its place, does not interfere with the subsequent application of the phenylhydrazine or picric acid tests, or the determination of the glucose by Pavy's solution. When the latter method is to be used, it is convenient to treat the filtrate from the precipitate produced by zinc with an equal measure of strong ammonia, which will ensure the zinc remaining in solution, and will furnish a constant supply of ammonia during the titration.

As pointed out by the author, in cases where the mere detection of glucose is sufficient, there is no occasion to remove the mercury from the solution. An excess of potassium iodide may be added, which will prevent precipitation on subsequently adding caustic alkali, and if the liquid be then boiled a grey precipitate of mercury will be produced if sugar be present. Unfortunately, this reaction cannot readily be made quantitative.

Reaction of Glucose with Copper Solutions.

The reduction of cupric compounds to cuprous by reaction in alkaline solution with glucose is one of the best known and deservedly popular methods of testing for sugar in urine. There are, however, several serious sources of error connected with the reaction, and ignorance or neglect of these has not unfrequently led to mistaken diagnosis and consequent improper medical treatment.

Solutions of the ordinary blue and green salts of copper on treatment with an alkali yield a pale blue precipitate of cupric hydroxide or hydrated

[1] Dr James Edmunds recommends that the cold liquid should be poured through a column of zinc-dust contained in a Mohr's burette (one-third filled) and retained in position by a plug of glass- or cotton-wool.

oxide of copper, having the composition CuO_2H_2, or CuO,H_2O. When the liquid containing this precipitate is boiled, the hydroxide loses its water of constitution and is converted into black cupric oxide, CuO. The cuprous salts, corresponding to cuprous oxide, Cu_2O, are mostly insoluble in water, and are readily converted into the blue cupric salts by oxidation. On addition of a caustic alkali to a cuprous salt, either in the solid state or in solution, a bright yellow cuprous hydroxide, CuOH, or Cu_2O,H_2O, is precipitated, and this, on boiling the liquid containing it, is dehydrated and converted into red or brownish-red cuprous oxide, Cu_2O.

The precipitation of cupric oxide from cupric salts by alkalies is prevented by the presence of tartaric acid, citric acid, glycerin, the sugars, and various other kinds of organic matter; but these substances exert no similar interfering action on the precipitation of cuprous oxide from cuprous solutions. Various oxidisable bodies convert cupric salts to cuprous, by assimilating one-half of the oxygen ($2CuO = Cu_2O + O$), and this occurs with special facility at the boiling point and in the presence of free caustic alkali. It follows, therefore, that if caustic potash or soda be added to a solution of cupric sulphate to which sufficient of an interfering agent has been added, no precipitate will be formed, the copper remaining in solution and communicating a deep blue colour to the liquid. If a body capable of abstracting oxygen from cupric oxide be now added, reduction to the cuprous state will ensue, and the yellow cuprous hydroxide or red cuprous oxide (according to the temperature of the liquid and other conditions of the experiment) will separate. With phenyl-hydrazine, reduction and consequent precipitation occurs immediately, and at the ordinary temperature. With glucose, it occurs slowly

in cold solutions, but immediately on boiling; while cane-sugar does not reduce the copper solution even at a boiling heat, although it shares with glucose the property of preventing the precipitation of cupric solutions by alkalies.

Upon the foregoing facts the various modifications of the copper test for glucose have been based.

TROMMER'S TEST.—If urine or other liquid containing glucose be treated with sufficient of a solution of copper sulphate to render the liquid greenish, and a solution of caustic potash or soda be then added, a deep blue liquid will be formed if sugar in notable amount be present. On allowing the liquid to stand for some hours in the cold, a yellow precipitate of cuprous hydroxide or red precipitate of cuprous oxide will be produced, and the same precipitate will be obtained immediately on boiling the liquid. Trommer's modification of the copper test is merely of qualitative value, and should be resorted to only in cases where the conditions make the application of Fehling's form of the test impossible.

FEHLING'S TEST.—In Fehling's form of the copper test for glucose, reliance is not placed on the sugar itself for the prevention of the precipitation of the cupric solution by an alkali, but sufficient tartrate of alkali-metal is added to form a double tartrate of copper and sodium, which is not decomposed by alkalies. One molecule of tartaric acid is able to prevent the precipitation of one molecule of copper by a caustic alkali, owing to the formation of the salt $(CHO)_2Cu(CO_2K)_2$, but it is better to employ twice the amount of tartaric acid requisite for the production of this body. Fehling's solution is therefore essentially a solution of the double tartrate of copper (cupric) and sodium or potassium, with a considerable quantity of caustic alkali. It is best prepared as follows:—(*a*)

34·64 grammes weight of pure crystallised sulphate of copper (free from iron and moisture) is dissolved in warm distilled water, the solution filtered if necessary, and when cold diluted to 500 c.c. with distilled water; (*b*) 180 grammes of crystallised Rochelle salt (potassium sodium tartrate) is dissolved in about 300 c.c. of hot water, the liquid filtered if necessary, and 70 grammes of good caustic soda or 100 grammes of caustic potash added to the filtrate, which is then cooled and made up to 500 c.c.

Fehling's Solution is prepared by carefully adding an accurately measured volume of the above copper sulphate solution to an equal measure of the alkaline tartrate mixture. The precipitate first formed dissolves on agitation with formation of a clear azure-blue solution.

Fehling's solution is affected by air and light, undergoing a change which causes a precipitation of cuprous oxide on boiling. The alteration is often attributed to the formation of the isomeric modification of tartaric acid known as racemic acid, but the evidence on which this statement is made appears very insufficient. It is, at any rate, an undoubted fact that Fehling's solution spoils by keeping, and hence the indication obtained with a particular sample of the reagent cannot be trusted unless the solution be proved to give no precipitate of cuprous oxide when diluted with an equal measure of water and boiled for a few minutes. The best plan is to store the cupric sulphate and alkaline tartrate solutions separately, and to mix them only when required for use; then proving by actual trial that the mixture suffers no reduction when diluted and boiled as just described.

Fehling's solution is reduced by the following bodies when their neutralised solutions are heated to boiling with it:—

Carbohydrates, &c.—Dextrose, lævulose, maltose, isomaltose, lactose, galactose, xylose, arabinose.

Alcohols, aldehydes, phenols, &c.—Acetaldehyde, valeraldehyde, chloral, chloroform, resorcinol.

Acids.—Gallotannic, glycosuric, glycuronic, indoxyl-sulphuric, hippuric, salicylic, trichloracetic, uric.

Bases.—Creatine, creatinine, xanthine, phenyl-hydrazine. (The last substance reduces Fehling's solution in the cold, with evolution of nitrogen.)

Fehling's solution is not reduced when boiled with neutral or alkaline solutions of the following bodies:—

Carbohydrates, &c.—Cane-sugar, raffinose, mannitol, dulcitol, dextrin, arabin, cellulose.

Alcohols, aldehydes, phenols, &c.—Alcohol, glycerin, benzoic aldehyde, salicylic aldehyde, phenol.

Acids.—Acetic, citric, benzoic, formic, gluconic, lactic, lactonic, mucic, oxalic, protocatechuic, sulphurous, sulphamido-benzoic ("saccharine"), tartaric.

Bases.—Urea.

Among the products of the action of Fehling's solution on glucose are said to be the following bodies:—(1) Acetic and formic acids: (2) Certain non-volatile acids, especially tartronic; an acid forming uncrystallisable salts; and an acid which is decomposed with formation of humus-like products on heating its alkaline solution: (3) A gummy substance (compare page 24).

Fehling's copper solution, when properly applied, affords one of the simplest and most popular means of detecting glucose in urine. The usual method of employing it is to remove albumin, if present, by heating to boiling the slightly acid or acidulated urine, and filtering from any precipitate (see page 106). The filtrate is then rendered distinctly alkaline to litmus paper by a few grains of sodium carbonate, heated to boiling, and filtered from the precipitate of earthy

phosphates, &c. Ten c.c. of recently prepared Fehling's solution[1] is heated to boiling in a test-tube, some platinum wire or a few fragments of clay tobacco-pipe or pumice being added to prevent bumping. When the liquid boils, 1 c.c. of urine, prepared as above described, is added, and the liquid boiled again for a few seconds. If sugar be present in abundance, as in a decidedly diabetic urine, a yellowish or brick-red opacity and deposit will be produced. If a negative reaction be obtained, traces of sugar are tested for by adding 7 to 8 c.c. of the prepared urine to the hot liquid, heating again to ebullition, and then setting the tube aside for some time. If no turbidity is produced as the liquid cools, the urine is regarded as either quite free from sugar or containing less than 0·2 per cent. If the proportion of sugar be moderate—that is, under 0·5 per cent.—the precipitation of the yellow or red cuprous oxide does not take place immediately, but occurs as the liquid cools, the appearance being somewhat peculiar. The liquid first loses its transparency, and passes from a clear bluish-green to an opaque light greenish colour. This green milky appearance is said to be very characteristic of dextrose, but it would be more correct to say that its appearance indicated the presence of some substance interfering with the normal reaction of sugar. The number of these interfering bodies is very considerable, including, as it does, uric acid, hippuric acid, glycuronic and glycosuric acids, xanthine, hypoxanthine, creatinine, indoxyl-sulphates, mucus, peptones, and the milk-sugar sometimes present in the urine of nursing women. Some of these sub-

[1] It is absolutely necessary that the Fehling's solution employed for the test described in the text should remain clear when diluted with its own volume of distilled water and boiled for a few minutes. The cupric sulphate and alkaline tartrate solutions should be kept separate, and the Fehling's solution made when required by mixing them in equal volumes. Compare page 59.

stances exert a reducing action on the copper solution, while others, such as xanthine, uric acid, peptones and pigments, modify the reaction by causing the formation of a greenish precipitate instead of the separation of yellow or red cuprous oxide as normally obtained.

Modified Fehling's Test.—The presence of such interfering bodies render the indications of Fehling's solution uncertain and difficult to interpret when only small quantities of sugar are in question. A great improvement in the precision and delicacy of the test, as applied to urine, without in any way adding to the time required or to the complexity of the manipulation, can be effected by operating in a manner devised by the author. This consists in a preliminary treatment of the urine with cupric sulphate or acetate, in neutral or slightly acid solution, whereby many of the constituents of urine are precipitated without in any way affecting any form of sugar.

Cupric sulphate yields little or no precipitate with normal urine in the cold, but on standing or boiling a whitish or pale green precipitate is thrown down, which has a tendency to darken if the heating be continued. If cupric acetate be substituted for the sulphate, or if sodium acetate be added to the cupric sulphate solution, this precipitation is much more complete, uric acid, xanthine, hypoxanthine, colouring matter, and albumin (if present) being entirely thrown down, and the phosphates and creatinine partially. The filtered liquid cannot be used for the phenyl-hydrazine test, and the presence of copper unfits it for titration by Pavy's solution, but it is admirably adapted for the detection of small quantities of glucose by Fehling's test. The procedure is as follows:—

From 7 to 8 c.c. ($= \frac{1}{4}$ oz.) of the sample of urine to be tested is heated to boiling in a test-tube, and, without separating any precipitate of albumin which

may be produced, 5 c.c. (about 80 minims) of the solution of cupric sulphate used for preparing Fehling's test is added. This produces a precipitate containing uric acid, xanthine, hypoxanthine, phosphates, &c. To render the precipitation complete, however, it is desirable to add to the liquid, when partially cooled, from 1 to 2 c.c. (20 to 30 minims) of a saturated solution of sodium acetate having a feebly acid reaction to litmus.[1] The liquid is next filtered. To the filtrate, which will have a bluish-green colour, 5 c.c. (80 minims) of the alkaline tartrate mixture used for preparing Fehling's solution is next added, and the liquid boiled for fifteen to twenty seconds. In the presence of more than 0·25 per cent. of sugar, separation of cuprous oxide occurs before the boiling-point is reached; but with smaller quantities precipitation takes place during the cooling of the solution, which becomes greenish, opaque, and suddenly deposits cuprous oxide as a fine orange-yellow precipitate.[2]

The foregoing description is intended to apply more especially to the examination of urine containing a comparatively small proportion of sugar, as it is in such cases that the interference of the co-occurring substances is most marked and objectionable. But the method is equally applicable to the examination of strongly diabetic urine. In such cases, however, the

[1] Sufficient acetic acid should be added to the sodium acetate solution to render it feebly acid to litmus paper. A saturated solution of sodium acetate keeps well, but weaker solutions are apt to become mouldy, and then possess the power of reducing Fehling's solution. Hence it is essential in all cases of importance to make a blank test by mixing equal measures of cupric sulphate solution, alkaline tartrate solution, and water, adding a little sodium acetate solution, and heating the mixture to boiling.

[2] The sudden separation of the precipitate during cooling is a phenomenon difficult of explanation. Operating as prescribed in the text on urine containing but a small quantity of sugar, the precipitate is of a bright orange-yellow colour; but when typical diabetic urine is under treatment, or a solution of pure sugar is employed, the cuprous oxide is thrown down as a bright brick-red or brown precipitate. The modified colour of the precipitate yielded by slightly saccharine urine appears to be due to the extractive matters present.

measure of urine taken should be reduced from 7 or 8 to 1 or 2 c.c. (= 15 to 30 minims), or even less, water being added to bring the volume up to 7 or 8 c.c.

It is evident that in this modification of the ordinary Fehling's test, advantage is taken of the very general precipitating power of cupric acetate to remove from the urine the great majority of those substances which interfere with the detection of diabetic sugar; and which by themselves either reduce the alkaline copper solution, retaining the cuprous oxide in solution, or produce a flocculent precipitate which masks the true reaction of sugar. Operating as above described, no greenish turbidity refusing to settle is produced, and hence the separation of any cuprous oxide is very readily observed.

In employing the foregoing modification of the copper test, it must not be forgotten that a solution of cupric acetate, even when acidulated with acetic acid, yields a precipitate of cuprous oxide when boiled with a solution of glucose. This reaction, first observed by Barfoed, and confirmed by Müller and the author, is liable to vitiate the test for small quantities of sugar, unless the prescribed method of working is strictly followed. Hence it is clearly important that the sodium acetate should not be added to the boiling hot solution, or the liquid boiled after its addition, or there will be danger of partial reduction by the sugar before adding the alkaline tartrate. But if the sodium acetate solution have a distinctly acid reaction, and its addition to the liquid be not made till the latter has somewhat cooled, the author has fully satisfied himself that there is no possibility of error from this cause.

A further practical advantage attending the employment of the foregoing modification of Fehling's test, is the complete precipitation of any albuminous

matters by the cupric sulphate solution without the necessity for an additional filtration; and the removal of these bodies at a prior stage prevents the formation of ammonia, which unavoidably ensues to a greater or less extent on boiling their solutions with caustic alkali. The formation of ammonia in this manner interferes with the detection of minute quantities of sugar by Fehling's test, since the cuprous oxide produced is held in permanent (colourless) solution.

The presence of any considerable quantity of ammonia, whether due to fermentation of the urea or other cause, absolutely invalidates the indications of Fehling's test, except, of course, when using Pavy's modification of it (page 66), in which a strongly ammoniacal solution of the copper reagent is intentionally employed.

On adding Fehling's solution to a solution of creatinine, a green liquid is produced, and on boiling this a yellow coloration is observed, without, however, any separation of cuprous oxide. In the case of urines rich in creatinine, this behaviour causes interference with the detection of glucose, the combination of the yellow and blue colours resulting in a green, and in addition the creatinine compound is said to have the power of preventing the precipitation of cuprous oxide by glucose. (See also the effect of dextrin observed by Reichard and described on page 31.)

In cases where the glucose present is more than equivalent to the cupric oxide in solution, as may happen when a urine containing a large proportion of sugar is being treated, the cuprous oxide precipitated often has a dark brown colour, while the supernatant liquid also has a brownish tint. This behaviour is due to the caramelisation of the excess of glucose by the caustic alkali, a portion of the colouring matter produced being carried down by the precipitate.

Volumetric Determination of Glucose by Fehling's Solution.—The last described modification of Fehling's test cannot be applied quantitatively, since an unknown amount of copper passes into the precipitate, while, owing to the insurmountable tendency, in many cases, of the cuprous oxide to pass through a filter, the gravimetric[1] and those modifications of the volumetric process in which filtration is necessary are not suitable for the determination of sugar in urine. In the absence of ammonia, either existing in the original urine or resulting from the decomposition of urea, fairly good results may be obtained by operating as follows:—10 c.c. of recently prepared Fehling's solution is diluted with 40 c.c. of recently boiled water, and the liquid heated to boiling in a porcelain basin. The urine, previously diluted with from 2 to 20 measures of recently-boiled water, is then gradually added from a burette, the liquid being kept gently boiling until the blue colour has nearly disappeared. The addition of urine is then continued more cautiously, removing the source of heat and allowing the cuprous oxide to subside after each addition, when, by inclining the basin, the slightest remaining blue tint can be perceived. When the decolorisation is complete, the volume of urine which has been used is noted. This measure contains 0·050 gramme of glucose.

Titration of Glucose by Pavy's Ammoniacal Cupric Solution.—For the examination of typical diabetic urine, containing a considerable proportion of sugar and in a perfectly fresh state, the foregoing process is fairly suitable, but it becomes troublesome

[1] It is a fact not generally recognised, but strongly urged on the author by Dr J. Edmunds, that the cuprous oxide separated in Fehling's test undergoes reoxidation with extreme facility, and to this behaviour is probably attributable the discordant results obtained by many observers who have tried to utilise the weight of the separated cuprous oxide as a means of estimating the glucose present.

or inapplicable where the amount of sugar is small and the extractives correspondingly high. In such cases, and indeed for general urine testing, it may be advantageously replaced by a process devised by E. W. Pavy (*Chem. News*, xl. 77), who employs an ammoniacal cupric solution prepared by mixing 120 c.c. of ordinary Fehling's solution with 300 c.c. of strong ammonia (sp. gr. 0·880), and with 100 c.c. of a 10 per cent. solution of caustic soda, or the same volume of a 14 per cent. solution of caustic potash.[1] The mixture is then made up with distilled water to 1 litre.

The oxidising value of Pavy's solution, prepared as above, is *one-tenth* that of Fehling's solution; *i.e.*, 50 milligrammes (= 0·050 gramme) of glucose reduce 10 c.c. of Fehling's or 100 c.c. of Pavy's solution.[2]

According to C. W. Purdy,[3] the ratio of reduction of Fehling's and Pavy's solutions by glucose varies according as they are prepared with caustic potash or caustic soda. The existence of such a difference has been disproved by direct experiments made in the author's laboratory. G. B. Brook found the oxidising action of Pavy's solution to be absolutely the same whether prepared with 56 of KHO, 40 of NaHO, or 56 of NaHO. In fact, the writer's experience is that the amount of caustic alkali and ammonia may be further varied very considerably without the oxidising value of Pavy's solution being appreciably affected.

When Pavy's solution is heated with a liquid containing glucose the cuprous oxide produced does not

[1] If Fehling's solution be not at hand, Pavy's solution may be made up directly as follows:—4·157 grammes of pure crystallised sulphate of copper is dissolved in about 100 c.c. hot water and the solution cooled; 21·6 grammes of crystallised Rochelle salt and 18·4 grammes of caustic soda (*or* 25·8 grammes caustic potash) are dissolved in hot water, cooled, and added to the copper sulphate solution. To the mixture is added 300 c.c. strong ammonia (sp. gr. ·880), and the whole made up to 1 litre.

[2] One molecule of glucose reduces to cuprous oxide 6 CuO existing in Fehling's solution, but only 5 CuO in Pavy's solution prepared as described in the text.

[3] *Practical Uranalysis and Urinary Diagnosis*, 1894.

separate as a precipitate, but remains in colourless solution. The progress of the reaction is therefore indicated by the gradual disappearance of the blue colour of the liquid, and when the reaction is complete—that is, when all the copper is reduced to the cuprous state—the liquid becomes perfectly colourless. But ammoniacal cuprous solutions are extremely oxidisable, and hence the blue colour indicative of

Fig. 4.—Apparatus for Pavy's Test.

the existence of a cupric compound returns rapidly on exposing the reduced liquid to the air.[1] It is necessary, therefore, to operate in such a manner as will ensure the complete exclusion of air during the process. This is best done by attaching the nose of the Mohr's

[1] J. Edmunds has pointed out, in a private communication to the author, that the cuprous oxide precipitated in Fehling's form of the test is also highly oxidisable, and he attributes the variable results obtained in the quantitative application of the test to insufficient recognition of this source of error.

burette containing the urine to a tube passing through the india-rubber stopper of a flask containing the copper solution, as shown in figure 4. A second tube conveys away the steam and ammoniacal gas, while, by means of a third tube, a slow current of coal-gas may be kept constantly passing throughout the operation.[1] The coal-gas may be dispensed with by allowing the exit-tube to pass into an empty Woulffe's bottle, which is connected with a flask containing cold water, as suggested by A. W. Stokes (*Analyst*, xii. 47). If the exit-tube be provided with a valve, the vapours may be passed direct into water without the intervention of a Woulffe's bottle. (See J. Steiner, *Chem. News*, xl. 139.) The author has suggested the employment of a layer of paraffin oil to prevent contact with the air, and the same proposal has been subsequently made by L. Peske.

It is a good plan to whitewash the iron plate which supports the flask, as by that means a marked contrast is obtained to the blue colour of the copper solution. Stokes supports the half of an opal gas-globe behind the flask (*Analyst*, xii. 47). H. M. Smith uses a flask the lower third of which is made of white opalescent glass[2] (*Chem. News*, lxxi. 165).

In carrying out Pavy's process, the author finds 25 c.c. of the copper solution a convenient quantity to employ. This measure of the ammoniacal cupric solution should be placed in the flask and a few fragments of pumice or tobacco-pipe added to prevent bumping. The tubes and burette are then adjusted, a slow current of coal-gas allowed to pass, and the contents of the flask brought to ebullition. The urine, preferably

[1] When coal-gas is employed, a brick-red film is formed on the surface of the liquid towards the end of the titration. This is not cuprous oxide, as might be supposed, but cuprous acetylide, C_2Cu_2,H_2O, formed from the acetylene invariably present in coal-gas.

[2] Obtainable from C. E. Müller & Co., 148 High Holborn, London, W.C.

freed from earthy phosphates by adding ammonia and filtering, is then gradually run in from the burette, the boiling being continued. The process is at an end when the blue colour of the liquid is wholly destroyed. The end-reaction is very sharply marked, but the reduction occurs more slowly than with the ordinary Fehling's solution, and hence the process must not be hurried or too low a result will be obtained. In the case of strongly diabetic urine it is desirable to dilute it to a definite volume with distilled water. On the other hand, when the proportion of sugar is small (*e.g.*, 0·2 per cent.), the volume of Pavy's solution may be conveniently reduced to 10 c.c. When 25 c.c. is the volume employed, the measure of urine required to decolorise it, multiplied by four, contains 0·050 gramme of glucose.

As Pavy's solution, like Fehling's original reagent, is reduced by uric acid, xanthine, creatinine, &c., as well as by glucose itself, it follows that this method also is apt to give inaccurate indications when only small quantities of urinary sugar are in question, or when an abnormally large proportion of one or more other reducing substances happen to be present. A series of experiments made in the author's laboratory by G. B. Brook showed that the interfering substances can be most readily and perfectly removed by a plan proposed by Maly and improved by G. Stillingfleet Johnson, which has been described at length on page 55. In practice, 60 c.c. of the urine to be tested should be treated with 15 c.c. of a cold saturated solution of mercuric chloride and 3 to 5 c.c. of a cold saturated solution of sodium acetate (see footnote on page 55). The liquid is boiled for five minutes, and filtered hot. The precipitate contains all the uric acid, xanthine, creatinine, phosphates, and albumin of the urine, and a portion of the urea. The preci-

pitate is washed twice with water, the filtrate acidulated with a few drops of acetic acid, boiled for ten minutes with zinc-dust,[1] and again filtered. The precipitate is washed, and the filtrate diluted to 120 c.c. with strong ammonia. This liquid has half the concentration of the original urine, and is added to not more than 50 c.c. of boiling Pavy's solution from a burette in the usual manner. The large excess of ammonia has no prejudicial influence on the result, while it keeps the zinc in permanent solution and ensures a constant but gradual supply of ammonia during the progress of the titration. The requisite washing of the mercuric and zinc precipitates can be avoided if 50 c.c. of the urine be boiled with powdered mercuric chloride and sodium acetate, the liquid filtered, the filtrate treated with zinc-dust and again filtered, and a known volume of the last filtrate mixed with an equal volume of strong ammonia.

Experiments made in the author's laboratory in the foregoing manner, by G. B. Brook, upon a limited number of samples of urine from apparently healthy persons, showed the purified liquid to have a reducing action upon Pavy's solution corresponding to the presence of from 0·05 to 0·13 gramme of glucose per 100 c.c. of the original urine, and yielded crystals of phenyl-glucosazone by the phenyl-hydrazine test (see page 85). Previously to the removal of the interfering substances by the mercury reagent, the same urines had a cupric oxide reducing power corresponding to from 0·1 to 0·3 gramme per 100 c.c., equal to 0·44 to 1·3 grain of glucose per ounce. The difference between the amounts of apparent sugar shown before and after clarification is an indication of the proportion of interfering substances present.

G. Stillingfleet Johnson finds normal urine to have

[1] Or by Edmunds' plan, described in footnote on page 56.

absolutely no reducing action after clarification by the mercury reagent. This implies that an infinitely large quantity of the filtrate resulting from this treatment could be added to a limited volume of Pavy's solution without the blue colour of the latter being destroyed. But as the reduction of the mercuric solution with separation of calomel, and the simultaneous oxidation of the glucose, commences after a few minutes' ebullition, it is conceivable that when mere traces of sugar are present they might be entirely destroyed at this stage of the process, and that the amount found in the filtrate would be merely that which had survived the treatment.[1]

Recognising the objections attaching to any process in which glucose is subjected to the action of a hot solution containing fixed caustic alkali, Allein and Gaud (*Jour. Pharm. Chem.*, [5], xxx. 305; and *Jour. Chem. Soc.*, lxviii. ii. 92) have recently proposed to employ a strongly ammoniacal solution of cupric sulphate for determining sugar. The process has been tried in the author's laboratory, but the reduction was found to occur so slowly that the method cannot be recommended.

Glycerol-Cupric Solutions.—In 1870, J. Löwe suggested the use of glycerin as a substitute for alkaline tartrate in preparing an alkaline cupric solution. He gave a recipe for preparing the solution from cupric sulphate, glycerin, and caustic soda, but preferred to employ freshly precipitated cupric hydroxide instead of the sulphate (*Zeits. anal. Chem.*, x. 452). More recently, a glycerol-cupric solution has been advocated by W. S. Haines (Chicago), who prepares it by dissolving 30 grains of crystallised

[1] Experiments made in the author's laboratory, to test this point, showed that the oxidation by mercuric acetate of glucose in very dilute solutions (0·05 to 0·10 per cent.) was very imperfect, and that 80 to 90 per cent. of the glucose survived boiling with the mercuric reagent for ten minutes.

copper sulphate in 240 grain-measures of distilled water, adding ½ troy-ounce (= 240 grain-measures) of glycerin, mixing thoroughly, and adding 5 troy-ounces (5·4 English fluid ounces) of *liquor potassæ*. In using this solution, it is recommended to boil about 1 drachm in a test-tube and then add two or three drops of the urine to be tested and again boil. In presence of much sugar a copious yellow or yellowish-red precipitate will be thrown down. If a negative result be obtained, a further addition of urine should be made, and the boiling repeated, but it is absolutely necessary that the total volume of urine employed should not exceed six or eight drops for 1 drachm of the glycerol-cupric solution, otherwise a precipitate may be produced even in the absence of sugar.

It is desirable previously to remove the earthy phosphates from the urine to be tested by adding a few drops of sodium carbonate and filtering. Even with this precaution, the author does not find the glycerin solution so delicate a test for sugar as is the ordinary tartrate solution of Fehling. But the glycerin mixture has the advantage of being more readily extemporised, and is said to keep indefinitely. Hence it deserves recognition as a convenient clinical test for diabetic sugar.

A glycerol-cupric solution of practically the composition of that prepared by Haines' formula may be obtained by mixing 2 measures of the cupric sulphate solution employed for preparing Fehling's test with 1 measure of pure glycerin, and then adding 10 measures of *liquor potassæ*, B.P.

C. W. Purdy (*Practical Uranalysis*, page 110) has proposed to employ Haines' glycerol-cupric solution with the addition of ammonia in place of Pavy's solution, but experiments on the process conducted in the author's laboratory showed that the method was

open to grave objections, and he is unable to recommend it.

GERRARD'S CYANO-CUPRIC PROCESS OF GLUCOSE-TITRATION.—A volumetric method of sugar titration, which will probably supersede all the other forms of copper determination of diabetic glucose, has been devised by A. W. Gerrard. The process, as originally published (*Year-Book Pharm.*, 1892, page 400, and *Pharm. Jour.*, [3], xxiii. 208), did not yield good results, but the writer has obtained further particulars from the inventor of the process, and has personally investigated the sources of error and the precise conditions necessary to ensure success. Gerrard's process is based on the following facts:—

When a solution of potassium cyanide is added to one of a cupric salt, a double cyanide of potassium and copper is formed, according to the following equation:—$CuSO_4 + 4KCy = CuCy_2,2KCy + K_2SO_4$. The double cyanide of potassium and copper thus formed is not decomposed by alkali nor precipitated by sulphuretted hydrogen. Its solution is colourless or faintly yellow. If Fehling's solution be employed instead of one of cupric sulphate, the same colourless double cyanide is formed, and the solution gives no precipitate of cuprous oxide when boiled with glucose solution. If Fehling's solution be present in quantity more than sufficient to react with the potassium cyanide used, this extra portion will suffer reduction by glucose; but instead of the cuprous oxide being precipitated it will remain in solution, and the progress and end of the reduction will be indicated by the gradual lessening and ultimate entire disappearance of the blue colour. Hence in outward appearance the method resembles that of Pavy (see page 67), but it has the advantage of causing no evolution of ammonia. Further, the re-oxidation of the reduced solution pro-

ceeds slowly, so that, where the highest degree of accuracy is not sought, the titration, if conducted expeditiously, may be performed in an open basin, instead of in an apparatus jealously protected from ingress of air. In its improved form, as modified by Gerrard and the author, the procedure is as follows:—

Ten c.c. of freshly-prepared Fehling's solution, or 5 c.c. of each of the constituent solutions, both accurately measured, should be placed in a porcelain dish, 40 c.c. of water then added, and the liquid heated to boiling. A solution of potassium cyanide, of about 5 per cent. strength, is gradually added from a burette or pipette,[1] until the deep blue colour of the liquid is nearly destroyed. The addition of the cyanide is continued, very cautiously, drop by drop, the liquid being kept constantly boiling and well stirred, until the blue colour just disappears, or only a very slight tinge of blue remains. Excess of cyanide solution must be carefully avoided, so that it is safer to discontinue its addition when the blue colour is still faintly perceptible.[2] Another 10 c.c. of Fehling's solution, or its equivalent, should now be added to the contents of the basin, and the urine or other sugar-holding

Fig. 5.—Fletcher's Pipette.

[1] Fletcher's graduated stopper-pipette (fig. 5) is very convenient for this and similar work. It has a small slit in the upper india-rubber ball which acts as a valve, and by judicious pressure the liquid in the pipette can be delivered drop by drop or rapidly as may be desired. The "*Automatic* Stopper" is manufactured by Messrs Fletcher, Fletcher, & Co., Holloway, London, N.

[2] There is no occasion to go through this process every time. The double solution of potassio-cupric cyanide keeps unchanged for some weeks, so that 100 c.c. of Fehling's solution may be diluted with about 300 c.c. of water, and the liquid decolorised while boiling by a 5 per cent. solution of potassium cyanide. The liquid is then diluted to exactly 500 c.c., and preserved in a well-closed bottle. Fifty c.c. of this solution, corresponding to 10 c.c. of Fehling, are mixed with 10 c.c. of untreated Fehling's solution, the liquid heated to boiling, and the titration conducted as described in the text.

liquid dropped in *rapidly* from a burette, with constant stirring, the liquid being kept in ebullition. The end-reaction is indicated by the disappearance of the blue colour, a point which can be very sharply observed when pure solutions of sugar are being treated, but which is somewhat less distinct when urine is under examination.[1] The volume of saccharine liquid required to decolorise the solution contains 0·050 gramme of glucose. Hence it follows that 10 c.c. of urine containing 0·5 per cent. of sugar will be required, and proportionately less of stronger solutions. Therefore urine suspected to contain more than 0·5 per cent. of sugar should be diluted with water to a definite measure before being used. For ordinary diabetic urine, 10 measures should be diluted to 100.

Of course, if preferred, grains or minims may be substituted for the metric weights and measures prescribed above. In such case, 100 grain-measures of Fehling's solution or its equivalent may be reduced by cyanide, and a second 100 grain-measures added. A. W. Gerrard has patented a special form of burette for use with the process.[2] This is so graduated as to allow the percentage of sugar contained in the urine to be read off at once without calculation.

For simplicity, accuracy, and general convenience, Gerrard's cyano-cupric process is preferred by the w r i t e r to any other method of titration based on the reducing power of glucose. No doubt the other reducing constituents of urine (*e.g.*, creatinine, uric acid, &c.) exercise the same disturbing influence on the results as in other methods, but they do not visibly interfere with the indications.

[1] Only the second measure of Fehling's solution suffers reduction. Experiments in the writer's laboratory, by Arnold R. Tankard, have proved that the reducing power of invert-sugar solution on the cyano-cupric reagent is securately as stated in the text.

[2] Obtainable from Messrs Gibbs, Cuxson, & Co., Wednesbury.

Reaction of Glucose with Bismuth Compounds.

A test for glucose which has the merits of simplicity and convenience, is the bismuth test due to Böttger. The urine to be examined is mixed with an equal measure of *caustic* potash or soda solution, and a small quantity (as much as can be taken up on the point of a pen-knife) of basic nitrate of bismuth added. On boiling the liquid, metallic bismuth will separate as a grey deposit or black metallic mirror if sugar be present in notable amount. Nylander has improved this test by substituting a solution of sodio-tartrate of bismuth for the solid oxy-nitrate employed by Böttger. 2·5 grammes of the basic nitrate of bismuth (free from silver) and 4 grammes of Rochelle salt are dissolved in 100 c.c. of an 8 per cent. solution of caustic soda. On boiling 10 c.c. of this reagent, filtered if necessary, with 1 c.c. of urine for a short time, the liquid will turn black if notable traces of glucose be present. Albumin, blood, and other sulphuretted bodies are liable to cause confusion by precipitating black bismuthous sulphide, and hence must be removed before the test is applied.[1]

A. Jolles (*Chem. Centr.*, 1890, ii. 609) states that with Böttger's test 0·08 per cent. of sugar can be detected in urine, and this limit is reduced to 0·01 per cent. if the urine be previously boiled with a solution of sodium chloride. At least 50 c.c. of the urine should be employed to 5 c.c. of Nylander's reagent, and the liquid should not be boiled more than two minutes. The presence of such substances as rhubarb, turpentine, kairine, quinine, sulphur, arsenic, mercury, iodides, and salicylic acid should be avoided. A brown colour is produced in presence of considerable

[1] Experiments in the author's laboratory showed that 1 c.c. of a 0·1 per cent. solution of glucose, when boiled with 10 c.c. of Nylander's reagent, gave a grey coloration, and a light grey mirror appeared on the tube. With 1 c.c. of a 1·0 per cent. glucose solution, the liquid became greenish-black, and a metallic mirror was formed on the tube.

quantities of uric acid, but the depth of tint from this cause does not increase beyond a certain point, whereas that due to sugar continually darkens.

Reaction of Glucose with Mercuric Compounds.

Several methods of determining glucose have been based on its reaction with mercuric compounds. Thus an alkaline solution of potassio-mercuric cyanide has been recommended by Knapp; an alkaline solution of potassium mercuric iodide ("Nessler's reagent") by Sachsse; and a solution of mercuric acetate by Hager. The mercury of the first two solutions is, on boiling, promptly reduced to the metallic state, and that of Hager's reagent more gradually to the mercurous condition. Hence in presence of chloride, as when the mercuric acetate is extemporised by mixing solutions of mercuric chloride and sodium acetate, a precipitate of mercurous chloride is formed on boiling the liquid for a short time (compare page 72).

The objections to the mercurial solutions are much the same as apply to those of copper, and they present no advantages to warrant an addition to the already numerous methods of determining diabetic sugar.

Reaction of Glucose with Organic Colouring Matters.

Picric Acid Test for Sugar.—Among the many coal-tar dyes and other organic compounds which suffer reduction in alkaline solution by glucose is the substance commercially known as "picric acid." This body has the constitution of a trinitro-phenol, and is obtained by the action of excess of strong nitric acid on carbolic acid:—

$$C_6H_5.OH + 3HNO_3 = C_6H_2(NO_2)_3.OH + 3H_2O.$$

Picric acid, which occurs in pale yellow crystals, sparingly soluble in cold water, but more readily in

alcohol, ether, and benzene, readily suffers reduction in hot alkaline solution to picramic acid, a body having the constitution of triamido-phenol :—

$$\underset{\text{Picric Acid.}}{C_6H_2(NO_2)_3.OH} + 9H_2 = \underset{\text{Picramic Acid.}}{C_6H_2(NH_2)_3.OH} + 6H_2O.$$

In practice, it is convenient to employ a cold saturated aqueous solution of picric acid, mixed with an equal measure of normal caustic soda or potash solution (or *Liquor potassæ*, B.P.).[1] The liquid is then boiled, and the urine or other liquid to be tested for glucose added, the boiling being repeated for a minute or so. If glucose be present, the solution will darken from the yellow-orange colour characteristic of a hot alkaline solution of picric acid to an intense brownish-red colour, so deep as to be opaque in presence of more than traces of sugar.[1]

This reaction, originally proposed as a test for sugar by C. D. Braun (1865), has been strongly advocated of late years by Sir G. Johnson, who has described a colorimetric application of it by which estimations of diabetic sugar may be obtained.[2] Picric acid is

[1] The picric acid can conveniently be mixed with the alkali before it is required for use. If caustic potash be employed, an immediate deposition of beautiful crystals of the sparingly soluble potassium picrate is produced, a mere fraction of the picric acid remaining in solution. If caustic soda be substituted, a similar effect is produced in cold weather, but the separation of the sodium picrate is by no means so prompt or complete as that of the potassium salt. Indeed, a permanent and serviceable reagent can be prepared by mixing two measures of cold picric acid with one of normal soda solution, disregarding any separation of crystals which may occur.

In either case it must be remembered that alkaline solutions of picric acid are much deeper in colour than those of free picric acid, and that on heating the colour becomes intensified. Impure picric acid gives a still deeper colour.

[2] Sir George Johnson compares the colour yielded by the sample of urine in question with a permanent standard solution prepared as follows :—

℞.—Liq. Ferri Perchlor. Fort. (B.P., sp. gr. 1·42),	. .	℥j.
Acid. Acet. Glacialis (B.P., sp. gr. 1·058),	. .	℥iv.
Liq. Ammoniæ (B.P., sp. gr. 0·959),	. .	℥vj.
Aquam Destill.,	. . . . .	ad ℥iv.

Mix the iron solution with the acid, then add the ammonia, and dilute with water up to 4 fluid ounces. This solution is of the same depth of colour as that produced by a solution of glucose containing 1 grain per fluid ounce, when

not affected by uric acid, but unfortunately creatinine gives with it a reaction very similar to that yielded by glucose. As a consequence, normal urine yields a blood-red colour when treated in the cold with an alkaline solution of picric acid, and the colour becomes more intense on boiling. This behaviour renders the positive detection of co-occurring traces of sugar very difficult. In fact, for use with untreated

boiled with picric acid and alkali. It should be preserved in a perfectly tight stoppered bottle, or preferably in the tube actually employed for comparing it with the glucose solution.

Sir G. Johnson employs for the comparison a tube about 12 inches long and 1 inch in diameter, graduated into 100 c.c., accurately stoppered and lipped. The standard solution is contained in a similar but shorter tube of exactly the same diameter, and accurately stoppered. These, under the name of "Johnson's Picro-saccharometer," can be obtained from C. E. Müller & Co., 148 High Holborn, London, W.C.

In the opinion of the author, Johnson's tubes are much wider than is desirable, far better results being obtainable by the use of the narrow graduated tubes employed for estimating carbon in steel by Eggertz' colour-test, and obtainable under that description of any apparatus-maker. Five c.c. measure of the sample of urine is treated with 5 c.c. of a saturated solution of picric acid and 2·5 c.c. of normal caustic soda or potash. The liquid is boiled for about a minute, cooled thoroughly, and made up to 20 c.c. with distilled water. Five c.c. of this liquid is poured into the graduated tube, which holds 25 or 30 c.c., and is graduated to 0·1 c.c. Five c.c. of the standard solution of ferric acetate is placed in the twin tube, and the colours of the two liquids compared by holding the tubes side by side up to a good light, and looking through them transversely. The comparison is facilitated by placing a piece of moistened filter-paper between the tubes and the light.

If the solution of the treated sample be lighter than the standard it may be concluded that the urine contains less than 1 grain of sugar per ounce. ("All normal urines reduce picric acid to an extent equivalent to ½ to 1 grain of glucose per fluid ounce. This reduction is due to creatinine, and should be allowed for when the proportion of glucose present is small."—*Sir G. Johnson.*) If, on the other hand, the solution of the sample be darker than the standard, the former must be diluted with distilled water until they are exactly of the same tint. If the sample require dilution to more than 30 c.c. in order to match the tint of the standard, the process must be repeated on the suitably diluted sample of urine.

The volume to which the treated urine requires to be made up, divided by five, gives the number of grains of glucose contained in each fluid ounce of the urine. Thus, if a sample of urine be diluted to four times its measure with water, and 5 c.c. of this, after treatment with picric acid, require to be diluted to 23 c.c. in order to match the standard, then :—

$$\frac{4 \times 23}{5} = 18{\cdot}4 \text{ grains of sugar per fluid ounce.}$$

This result, divided by 4·375, gives 4·26 per cent. of sugar in the urine.

urine, the test is not, in the opinion of the author, so useful or trustworthy as that with safranine, where the reaction consists in the discharge of a deep red colour by sugar, instead of in its production. Further, the safranine test is not affected by the constant presence of creatinine. If the creatinine be previously removed by mercuric acetate, as recommended by G. S. Johnson, and the mercury subsequently entirely removed from the filtrate, as described on page 70, the picric acid test will indicate very minute quantities of glucose, and very fair colorimetric estimations may be made in the liquid thus clarified.

METHYLENE-BLUE REACTION FOR GLUCOSE.—The coal-tar colouring matter known in commerce as *methylene-blue* is the hydrochloride or double zinc salt of a base having the formula $C_{16}H_{18}N_3S.OH$, and the constitution of a tetramethyl-thionine hydroxide. By the action of reducing agents, including a boiling alkaline solution of glucose, this body is reduced to tetramethyl-leucothionine, $C_{16}H_{19}N_3S$, a colourless substance which undergoes oxidation with extreme facility, forming methylene-blue and other products. N. Wender (*Pharm. Post.*, xxvi. 93, and *Analyst*, xviii. 131) has proposed to utilise this reaction for the recognition and rough estimation of diabetic sugar. The test has been fully tried in the author's laboratory, and the following mode of employing it is substantially that prescribed by Wender:—

A solution of methylene-blue is prepared by dissolving 1 part of the commercial colouring matter in 3000 parts of distilled water (=0·333 gramme per litre). Six c.c. (=90 minims) of this solution is first measured into a test-tube, and 2 c.c. (=30 minims) of a normal solution of caustic potash (or *Liquor potassæ*, B.P.) added. The sample of urine to be tested is then

diluted to ten times its bulk with water, and 2 c.c. (=30 minims) of the diluted urine added to the alkaline solution of methylene-blue. The contents of the test-tube are then boiled for a minute or two, avoiding agitation or contact with air as much as possible, when complete discharge of the blue colour will ensue if the sample of urine contained 0·5 per cent. or more of sugar. By repeating the test with 4 c.c. or still larger volumes of the diluted urine, smaller proportions of glucose may be approximately estimated. A good plan is to immerse in a bath of boiling water four or more test-tubes, each containing 6 c.c. of the methylene-blue solution and 2 of caustic alkali, to which gradually increasing measures of urine have been added, when a close indication of the amount of sugar can be obtained in the time requisite for completing a single test. Thus, if the blue colour is not discharged by 4 c.c. of the diluted urine, but is destroyed in the tube to which 6 c.c. has been added, the proportion of sugar present is less that 0·25, but greater than 0·17 per cent.

The outward similarity of the reaction of glucose with Pavy's solution to its behaviour with alkaline methylene-blue, suggested to the author the possibility of employing the latter reagent in precisely the same manner as the ammoniacal copper solution. A series of experiments in this direction, made in the author's laboratory by G. B. Brook, have shown, however, that methylene-blue presents several practical disadvantages. The discharge of the colour is not so prompt nor the end-reaction so distinct as in the case of Pavy's solution. The blue colour returns with the utmost facility, a reduced solution of methylene-blue being in fact one of the most delicate existing tests for free oxygen. The employment of a layer of paraffin oil is not feasible, since the free base of the

colouring matter is liberated by the alkali and dissolves in the oil with red colour, and, of course, is prevented from free reaction with the glucose, while on the other hand it is exposed to the air.[1]

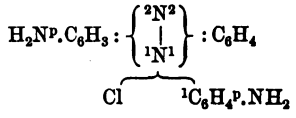
As a clinical test for diabetic sugar, the methylene-blue reaction is very serviceable. The discharge of the colour is a phenomenon far more readily observed, and requires less judgment, than the distinction between different shades of red and brownish-red required in applying the picric acid test.[2]

SAFRANINE TEST FOR GLUCOSE. Various other coal-tar dyes are reduced and decolorised by heating with glucose in alkaline solution. One of these, safranine, recommended by M. Crismer (*Pharm. Zeit.*, xxxiii. 65; *Pharm. Jour.*, [3], xix. 348), is especially suitable for the purpose. Safranine occurs in commerce[3] as a brownish-red powder, readily soluble in water to form a blood-red solution not liable to change by keeping. No immediate precipitate is produced on adding caustic alkali solution, but on standing more or less separation occurs. On adding to the alkaline liquid a solution of glucose, and heating it to the boiling-point, the liquid is changed to a pale urine-yellow,

[1] It is possible that the red substance dissolved by the paraffin oil is some extraneous impurity, but the same behaviour occurs when ether is substituted, and on separating the ethereal layer and shaking it with acid, a blue colouring matter passes into the latter. Attempts to purify the commercial methylene-blue by precipitating the colouring matter by potassium iodide or brine did not affect its subsequent behaviour with paraffin oil.

[2] The writer found approximately 5 per cent. of sugar by the methylene-blue test in a sample of diabetic urine, which, by more accurate analysis, was proved to contain 4·9 per cent. In this case 1 part of the urine was diluted to 100 by water.

[3] Commercial safranine is a mixture of several homologous bodies, of which $C_{19}H_{17}N_4Cl$, $C_{20}H_{19}N_4Cl$, and $C_{21}H_{21}N_4Cl$ are the chief. The second of these has the constitution of a chloride of paramidophenyl-paramido-phenazonium, and the following structural formula :—



and becomes turbid from the separation of the insoluble leuco-derivative. If the glucose be not present in large excess, the red colour returns on agitating or exposing the solution to the air.

Safranine is not decolorised when heated in alkaline solution with uric acid, creatine, creatinine, chloral, chloroform, hydrogen peroxide, or salts of hydroxylamine, and only slowly (but in the end completely) by solutions of albumin. Hence any discharge of colour produced by a sample of urine, preferably freed from albumin, may confidently be set down to the presence of sugar. The indifference of safranine to uric acid and creatinine has been fully verified by the author, who employs the reagent in the following manner :—Equal measures (2 c.c., or 30 minims) of the urine, normal soda or potash (or *liquor potassæ*, B.P.), and a solution of one part of safranine in 1000 parts of water are mixed. The liquid is heated in a test-tube till it boils freely, agitation being avoided as much as possible. If the urine contain more than 0·1 per cent. of sugar the liquid will be decolorised, but otherwise the red colour will remain intact or be only partially discharged. If the colour be destroyed, the test may be repeated with twice or three times the volume of safranine solution that is used of urine, every additional measure of safranine which is found to be decolorised representing roughly 0·1 per cent. of sugar in the sample. If more than four or five measures are required, the urine is distinctly diabetic, and it is desirable to dilute a portion to ten times its bulk with water, and employ equal measures of this diluted urine, alkali, and safranine solution. Pellets containing a definite weight of safranine may be used.

The safranine test has been fully investigated in the author's laboratory by G. Bernard Brook. It is one of the simplest and most certain clinical

tests for glucose, and deserves to come into general use for that purpose. It possesses the advantage over methylene-blue or picric acid of not being affected by uric acid or creatinine, while its application is quite as simple as that of either of these reagents. The rough quantitative indications, obtainable as above indicated, will often afford great assistance to the medical practitioner. On the other hand, attempts to base on the reaction an accurate quantitative method, by operating in the manner of Pavy, have been unsuccessful.

Reaction of Glucose with Phenyl-Hydrazine.

As all the methods of detecting sugar in urine which are based on the reducing action of glucose are more or less vitiated by the presence of other reducing bodies, a special reagent for glucose has an exceptional value. This reagent exists in phenyl-hydrazine, which the classical researches of E. Fischer have shown to react with all bodies of the aldehyde and lactone classes, including glucose, with formation of well-defined compounds which are generally readily crystallisable, and insoluble, or only very sparingly soluble, in water. Thus, with a single molecule of phenyl-hydrazine, dextrose reacts to form dextrose-phenyl-hydrazine, $C_{12}H_{18}N_2O_5$, a body which forms fine crystals fusing at 144° to 145° C. But with excess of phenyl-hydrazine, a body of the osazone class is formed, dextrose yielding phenyl-dextrosazone, $C_{18}H_{22}N_4O_4$. Identically the same osazone appears to be formed from lævulose, so that it may be conveniently called phenyl-glucosazone. Similar compounds, differing in their melting-points, are yielded by maltose and lactose, but not by cane-sugar. The following table exhibits the distinguishing characters of such osazones as are interesting in connection with the examination of diabetic urine :—

Sugar, &c.	Compound (Osazone) with Phenyl-hydrazine.		
	Formula.	Appearance.	Melting-point, ° C.
Dextrose, . . Lævulose, . .	$C_{18}H_{22}N_4O_4$	Needles.	204 to 205.
Maltose, . .	$C_{24}H_{32}N_4O_9$	Tables.	190 to 191.
Lactose, . .	$C_{24}H_{32}N_4O_9$	Wide prisms.	200.
Cane-sugar, . .	No compound.	...	...
Glycuronic acid, .	$C_{42}H_{48}N_{10}O_{10}$	Yellow needles or brown resinous mass.	115 to 115.

In applying Fischer's reagent in practice, the phenyl-hydrazine is most conveniently used as the hydrochloride, a salt which forms small, thin, lustrous plates, of the formula $C_6H_5.HN.NH_2,HCl$. The crystals are sparingly soluble in cold water, more readily in hot. The salt is almost completely precipitated from its aqueous solution by strong hydrochloric acid, which behaviour furnishes a means of readily purifying it from aniline hydrochloride and other impurities. Much of the phenyl-hydrazine hydrochloride of commerce has a brownish tint, and should be purified in the manner above indicated, followed by re-crystallisation from hot water.[1]

To apply the phenyl-hydrazine test, R. von Jaksch (*Zeits. clin. Med.*, xi. 20) recommends that 50 c.c. of the suspected urine, previously freed from albumin, should be treated with 2 grammes of sodium acetate and from 1 to 2 grammes of phenyl-hydrazine hydrochloride,[2] and the liquid heated to 100° C. for

[1] C. W. Purdy states that phenyl-hydrazine hydrochloride must be handled with great caution, as its contact with the skin is liable to produce eczema of a malignant type.

[2] Or 2 oz. of urine, 40 grains of sodium acetate, and from 20 to 30 grains of phenyl-hydrazine hydrochloride.

half an hour. On cooling, the phenyl-glucosazone separates as an amorphous or crystalline precipitate of a yellow or brick-red colour. If amorphous, the precipitate should be dissolved in hot alcohol, and the solution diluted with water and boiled to expel the greater part of the alcohol, when the glucosazone will separate at once, or on cooling, in the form of characteristic yellow needles, which melt at 205° C. with decomposition. Phenyl-glucosazone is nearly insoluble in cold water or alcohol, more soluble in hot water, and moderately soluble in hot alcohol. It is dissolved by glacial acetic acid to form a lævo-rotatory solution.

Instead of operating in the manner prescribed by von Jaksch, the phenyl-hydrazine test may be applied in the following simple manner, which is substantially that recommended by C. Schwartz (*Pharm. Zeit.*, xxiii. 465) :—Ten c.c. of the urine is heated to boiling and treated with half its volume, or a sufficiency, of a 10 per cent. solution of neutral lead acetate. The liquid is boiled and filtered hot. Solution of caustic soda, in amount sufficient to redissolve the precipitate which first forms, is added to the filtrate, and then a little (as much as will lie on the point of a pen-knife) phenyl-hydrazine hydrochloride is dropped in. The liquid is then boiled for some minutes, and then strongly acidulated with acetic acid. In presence of much sugar, an immediate yellow turbidity or precipitate will be formed, but if only minute traces be present a yellow coloration is first produced, which, on cooling and standing, changes to a turbidity. In all cases, considerable time is required for the complete separation of the glucosazone, but the qualitative indication is readily and quickly obtained.

Unfortunately, the phenyl-hydrazine test does not appear susceptible of being applied quantitatively,

though, of course, the intensity of the reaction and the amount of precipitate afford a fair indication of the proportion of sugar present. As little as 0·005 per cent. ($=\frac{1}{40}$ grain per ounce) of glucose can be detected in urine by the phenyl-hydrazine reaction.

Uric acid, urea, xanthine, and creatinine in no way simulate the reaction of glucose with phenyl-hydrazine. The only bodies which can cause confusion are glycuronic acid and its derivatives (page 38). Glycuronates give with Fischer's reagent a yellow, crystalline compound resembling phenyl-glucosazone, but melting at 150°. When the osazone is produced in an acid solution, as in von Jaksch's modification of the test, the glycuronic acid compound is precipitated as a dark brown resinous mass, in which form it can cause no confusion. According to Hirschl (*Zeit. physiol. Chem.*, xiv. 377), in applying the phenyl-hydrazine test to urine, the liquid should be heated on the water-bath for at least an hour. The fine, bright yellow needles of phenyl-glucosazone, melting at 204° C., are readily distinguished from the brownish amorphous compound, melting at 150°, produced by glycuronic acid; but if the heating be not continued for the prescribed time, the latter compound may be deposited in a crystalline state, and be mistaken for glucosazone. Hirschl believes that traces of glycuronic acid compounds are normally present in human urine.

In all doubtful cases the indications furnished by the production of a turbidity or precipitate with phenyl-hydrazine should be confirmed by obtaining the osazone in a crystalline form, and examining it under the microscope. If possible, the melting-point should be observed, and in making this determination it is essential that the heating should be conducted as rapidly as possible, as the compound readily undergoes decomposition at a temperature approaching its melt-

ing-point. The coloration obtained on moistening a crystal of the glucosazone with a drop of concentrated sulphuric acid is also an important means of identification. Glucosazone acquires an olive-green colour, and on subsequently adding a minute quantity of manganese dioxide a fine purple colour is obtained, closely resembling that yielded by strychnine under similar treatment, but not undergoing the subsequent change to cherry-red which characterises the alkaloid (A. H. Allen).

The author has observed that phenyl-glucosazone is readily dissolved by ether from its acidulated aqueous solutions. On separating and evaporating the ether, the glucosazone can be dissolved in alcohol, and crystallised by adding water and evaporating, as already described.

According to Roos, human urine always gives an affirmative reaction with the phenyl-hydrazine test, and the same is said to be true of dogs' urine, while the excretion of rabbits gives especially well-formed crystals. By the application of the phenyl-hydrazine test to a limited number of samples of urine from persons apparently in perfect health, the author satisfied himself of the presence of minute traces of sugar in each case.

According to von Jaksch, no sugar can be detected by the phenyl-hydrazine test in the urine of persons poisoned by arsenic, potash, or sulphuric acid, but it always indicates the presence of sugar in the urine of those poisoned by carbonic oxide or other irrespirable gases. It is not improbable that the inhalation of the products of the imperfect combustion of coal-gas, as evolved from the faultily constructed gas-stoves now so commonly used, may account for some of the cases in which traces of sugar have been found in the urine of persons apparently in perfect health.

Reaction of Glucose with Benzoyl Chloride.

If a solution of glucose be shaken with a little benzoyl chloride, $C_6H_5.COCl$, white crystals of a compound of the two bodies gradually separate. Wedinski has employed the reaction to demonstrate the existence of traces of glucose in normal human urine; but the behaviour of co-occurring substances must be studied more fully than has hitherto been done before the indications of the test can be fully accepted.

ACETONURIA.

The later stages of diabetes are generally characterised by the appearance in the urine of certain substances which are evidently products of incomplete oxidation. Among these are hydroxybutyric acid, acetone, and aceto-acetic ether. These bodies probably have their origin in the proteids of the food, for their amount is independent of any addition of carbohydrates to the diet but becomes greater with increased proteid metabolism. They do not occur in all cases of diabetes, but generally in the more severe forms of the disease, in which the destructive metabolism of proteids is augmented. In the last stage of diabetes, when coma sets in, the amount of hydroxybutyric acid increases, while that of acetone diminishes.

The occurrence of hydroxybutyric acid, aceto-acetic ether, and acetone is not confined to diabetes, but has been observed in many other maladies, including fever, however caused; cancer, under certain conditions, and in acetonæmia, apparently produced by ingestion of alcohol. Hence the formation of acetone and the allied anomalous products of metabolism may not improbably be a consequence of certain complications which accompany diabetes, and not of the disease

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itself. The occurrence of aceto-acetic acid or its ethyl ester has been noticed in the urine of healthy persons after prolonged fasting, and minute traces of acetone exist in normal urine. The usual proportion of acetone excreted is stated to be 0·010 gramme per diem, but this is said to be increased to 0·500 gramme per diem, or even more, in marked cases of acetonuria.

The relationship existing between acetic acid, aldehyde, acetone, aceto-acetic acid, and hydroxybutyric acid is shown by the following formulæ :—

Acetic acid, ...	$CH_3—CO—OH$.
Aldehyde, ...	$CH_3—CO—H$.
Acetone (dimethylketone), .	$CH_3—CO—CH_3$.
Aceto-acetic acid (diacetic acid),	$CH_3—CO—CH_2—CO.OH$.
Hydroxybutyric acid, . .	$CH_3—CH(OH)—CH_2—CO.OH$.

Acetone. Dimethyl-ketone. $CH_3.CO.CH_3$.

Acetone results from the dry distillation of acetates, especially those of barium and calcium, and may be obtained in various other ways. It is a colourless, limpid liquid of peculiar ethereal odour and burning taste. It is miscible in all proportions with water, alcohol, and ether, but is insoluble in a *saturated* solution of calcium chloride.

Acetone has many of the characters of the aldehydes, and like them unites with the acid sulphites of the alkali-metals to form crystalline compounds. On adding to a tolerably strong solution of acetone a cold saturated solution of bisulphite of sodium, the soda compound separates out in shining scales.

When treated in solution with diazobenzene-sulphonic acid and a little sodium amalgam, acetone yields a dark red coloration. Aceto-acetic ether behaves similarly, while aldehyde gives a violet colour.

Baeyer and Drewsen have proposed a test for acetone based on the formation of indigo-blue. A few crystals of the highly explosive ortho-nitro-benzalde-

hyde are dissolved by the aid of heat in the suspected urine. On cooling, the reagent separates as a white cloud. The liquid is now made alkaline with dilute caustic alkali, when a yellow colour, changing in a few minutes to green and indigo-blue, will be developed if acetone be present.

The test is more satisfactory and delicate if applied to the first portion of the distillate from the suspected urine. To this, a solution of the nitro-benzaldehyde should be added, and the liquid made alkaline. If the change of colour from yellow to green and blue occurs tardily, the liquid may be shaken with chloroform, which dissolves the nascent colouring matter.

Acetone reduces Fehling's solution. If the test be applied to a distillate from the urine, of course all interference from glucose and other fixed reducing agents will be avoided.

P. Chautard (*Bull. Soc. Chem.*, xlv. 83) recommends for the detection of acetone that 200 c.c. of urine should be distilled at a gentle heat, and the first 15 c.c. of the distillate treated with a solution of rosaniline acetate previously decolorised with sulphurous acid.[1] If acetone be present, a fine reddish-violet coloration is produced, the intensity of which varies with the amount. It is stated that less than 0·01 per cent. of acetone can be detected in urine in this manner.

Villiers and Fayolle (*Bull. Soc. Chem.*, 1894, page 693, and *Jour. Chem. Soc.*, lxvi. 2, page 489) state that the crimson reaction with the rosaniline reagent

[1] The reagent is prepared by mixing 30 c.c. of a solution of magenta (rosaniline hydrochloride) containing 1 gramme per litre; 20 c.c. of a solution of sodium bisulphite of 1·31 sp. gr.; 3 c.c. of strong sulphuric acid and 200 c.c. of water. Villiers and Fayolle prepare their reagent by adding to a solution of magenta such an amount of sulphurous acid as will just decolorise it after long standing in a closely stoppered bottle. This reagent is very sensitive to all oxidising agents. It gives a red colour even on prolonged exposure to air. Glucose, galactose, and invert sugar give a coloration as intense as aldehyde, but lævulose, cane-sugar, maltose, and lactose give no reaction until the lapse of some days.

is not produced by pure acetone, but is due to aldehyde, which they state is almost always formed simultaneously with acetone. It seems probable that the reaction is common to both bodies, but is capable of detecting a much smaller amount of aldehyde than of acetone, since Mohler found that 0·01 gramme per litre of aldehyde but not less than 0·5 gramme of acetone could be detected in spirits by this test.

For the detection of acetone in urine, Legal (*Jour. Pharm.*, [5], xviii. 206) adds some drops of a concentrated solution of sodium nitroprusside, and makes the liquid slightly alkaline with caustic potash. When the red coloration first produced has disappeared, a little acetic acid is added, which in presence of acetone will produce a deep violet coloration.

A somewhat modified form of the above test has been described by Le Nobel (*Chem. Centralb.*, 1884, page 626). He adds to the urine a solution of sodium nitroprusside, previously made alkaline by caustic soda and so dilute as to have only a slight red tint. If acetone be present, even to the small extent of 0·0002 gramme, a ruby-red colour will be produced, changing in a few moments to yellow, and on acidulating the liquid and boiling, a greenish-blue or violet colour is developed.[1]

The most delicate test for acetone is that based on its ready conversion into iodoform when treated with caustic alkali and a solution of iodine. The following equation represents the final reaction, but the highly unstable potassium hypoiodite is probably formed as a first product.

$$\underset{\text{Acetone.}}{C_3H_6O} + 4KOH + 3I_2 = 3KI + \underset{\text{Acetate.}}{KC_2H_3O_2} + \underset{\text{Iodoform.}}{CHI_3} + 3H_2O.$$

To apply the iodoform test, 10 c.c. of the suspected

[1] The nitroprusside test has been applied very successfully by A. Ashby to the detection of acetone in methylated spirit and liquids containing it. The orange-yellow colour given by aldehyde can scarcely be mistaken for the far more intense colour produced by acetone.

liquid should be treated with five or six drops of a 10 per cent. solution of caustic potash or soda, and the liquid warmed to about 50° C. A solution of potassium iodide, fully saturated with free iodine, is next added drop by drop, with agitation, until the liquid becomes permanently yellowish-brown, when it is carefully decolorised by a further cautious addition of the caustic alkali solution. Any iodoform will now be apparent as a yellowish turbidity, gradually depositing at the bottom of the liquid as a yellow dust, which, when examined with a lens, is seen to be crystalline. Under a power of 300 diameters, its appearance is very characteristic, the usual forms being hexagonal plates, stars, and rosettes. The formation of iodoform is pre-eminently a test for acetone, but it is not peculiar to that substance, being also produced by alcohol and its homologues, aldehyde, various ethers, turpentine, sugar, &c. On the other hand, it is not given by pure methyl or amyl alcohol, chloroform, chloral, ether, or glycerin; by acetic, formic, or oxalic acid; nor by acetal till after the addition of acid. With acetone the reaction occurs immediately and in highly dilute solutions, whereas alcohol and most of the other above-named bodies are converted into iodoform only slowly and in concentrated solutions.

For the quantitative determination of acetone in urine by the iodoform reaction, H. Huppert (*Zeits. anal. Chem.*, xxix. 632) recommends a method devised by Messinger (*Berichte*, xxi. 3366). To get rid of interfering bodies, such as phenol, ammonia, and nitrous acid, 100 c.c. of the urine should be previously distilled with 3 c.c. of acetic acid, the distillate redistilled with 1 c.c. of dilute sulphuric acid, and if nitrous acid be present a third distillation with some urea is directed.

Messinger's method for the estimation of acetone, as modified by Collischonn (*Zeits. anal. Chem.*, xxix. 562), is as follows :—Twenty c.c. of normal caustic soda, free from nitrites, is added to the distillate obtained as above, and 30 c.c. of $\frac{1}{5}$ normal iodine solution added with constant agitation. The shaking is continued for four or five minutes, while the liquid is warmed to a temperature not exceeding 60° C. The liquid is then acidified with dilute hydrochloric acid (sp. gr. 1·025), an excess of decinormal sodium thiosulphate added, and the solution finally titrated back with the iodine solution, starch being used as an indicator.

Every 762 parts of iodine which enter into combination represent 70 parts of acetone previously present.

Instead of estimating the iodoform volumetrically, the excess of iodine may be got rid of by adding thiosulphate solution of unknown strength, and the iodoform extracted by agitating the liquid with ether, which is then separated, allowed to evaporate spontaneously, and the residue of iodoform weighed. 142 parts of iodoform represent 70 of acetone. The result is liable to be low, owing to the volatility of iodoform even at ordinary temperatures.

A. Schwicker (*Chem. Zeit.*, xv. 914) employs ammonia instead of fixed caustic alkali in making the iodoform test. Alcohol then in no way interferes, but aldehyde must be absent. The first fraction of the distillate from the suspected urine is mixed with a few drops of strong ammonia, and a few drops of decinormal solution of iodine then added. A black precipitate of nitrogen iodide is first formed, but this disappears on shaking and warming the liquid. If acetone be present in not too small a quantity, iodoform now makes its appearance, and increases on

adding a little more iodine. The characteristic odour is not greatly obscured by that of the ammonia. If the nitrogen iodide does not readily disappear, it may be got rid of by cautiously adding a few drops of very dilute sodium thiosulphate (hyposulphite) solution. If only traces of acetone are present, more time should be allowed before removing the nitrogen iodide, and the iodine solution used should be very dilute. One part of acetone in 5000 of water is said to be recognisable by this modification of the test. Together with iodoform, there is formed a substance which is characterised by a pungent smell and the property of exciting lachrymation. It is probably an iodine-substitution-product of acetone.

E. Salkowski states that sugar, when present in urine, gives rise to a substance of an aldehydic nature (probably acetaldehyde), which on distillation simulates many of the reactions of acetone, including the formation of iodoform.

The breath and urine of persons in which acetone can be found by the iodoform and other chemical tests often have an odour of acetone. Though the occurrence of acetone in the urine often precedes the coma which ushers in the last stage of diabetes, acetone is not itself a toxic agent. Given in large doses to men and animals, even when diabetic, it produces no coma, and it is met with in many febrile diseases in which no coma occurs.

Von Jaksch found minute traces of acetone in the urine of healthy persons, the amount excreted being in some cases as high as 0·01 gramme (= 0·15 grain) in the twenty-four hours. For its detection in such minute quantities a large quantity (*e.g.*, 10 gallons) should be acidulated with dilute sulphuric acid and distilled, the first fractions redistilled, and the distillate saturated with fused calcium chloride, which

unites with the alcohol while leaving the acetone unchanged. The liquid is distilled on the water-bath, the distillate again treated with calcium chloride, and the process repeated until the acetone is concentrated in a comparatively small bulk of fluid, when it may be detected and determined by the methods already detailed.

Aceto-Acetic Acid. Diacetic Acid. Acetone-Carboxylic Acid. $CH_3.CO.CH_2.COOH$.

This body has been obtained synthetically by treating aceto-acetic ether in the cold with a solution of an equivalent quantity of caustic potash, and after twenty-four hours acidifying the liquid and agitating with ether, which extracts the free aceto-acetic acid. On evaporation, the acid is obtained as a syrupy liquid, miscible with water and decomposing at 100° C. into acetone and carbon dioxide :—

$$CH_3.CO.CH_2.COOH = CH_3.CO.CH_3 + CO_2.$$

Aceto-acetic acid and its salts give a violet or claret-red coloration with ferric chloride. The *barium* salt is amorphous and very soluble in water. The *copper* salt is also amorphous.

Ethyl Aceto-acetate, *Aceto-acetic Ether*, or *Diacetic Ether*, is the ethyl ester of aceto-acetic acid. Its composition is $CH_3.CO.CH_2.CO.O(C_2H_5)$. It is prepared by the action of sodium on perfectly dry ethyl acetate. Aceto-acetic ether is a liquid of an agreeable fruity odour, having a specific gravity of 1·0317 at 15° C., compared with water at the same temperature. It boils at 181° C., is slightly soluble in water, and the solution gives a violet or claret coloration with ferric chloride. Aceto-acetic ether resembles aldehydes and ketones in combining with the acid sulphites of the alkali-metals to form crystalline compounds, which are decomposed into their constituents by treatment with

sodium carbonate. The formation of the compound with acid sodium sulphite may be conveniently used for purifying the ether.

Aceto-acetic ether, like acetone, gives, with diazo-benzene-sulphonic acid and a little sodium amalgam, a dark red coloration.

Aceto-acetic ether behaves like a monobasic acid, and forms salts. The sodium compound is produced by the action of sodium or sodium ethylate on aceto-acetic ether in the cold, and has the following formula :— $CH_3.CO.CHNa.CO.O(C_2H_5)$.

By the action of alkalies, aceto-acetic ether undergoes decomposition into acetone, alcohol, and carbonic acid. The sodium salt reacts similarly, the change in this case being represented by the equation :—

$$CH_3.CO.CHNa.CO.O.C_2H_5 + 2H_2O = CH_3.CO.CH_3 + H.O.C_2H_5 + NaHCO_3.$$

This decomposition probably occurs in the blood and urine in cases in which acetone is present.

The presence of aceto-acetic acid or its derivatives in urine is an unfavourable symptom often preceding the onset of diabetic coma and death. Such urine often has an odour of acetone, and gives a violet or claret-red coloration with ferric chloride. The colour is destroyed by boiling, and is not produced at all if the urine has been boiled previously. Ferric chloride produces a somewhat similar coloration in the urine of persons who have taken salicylic acid, phenol, antipyrine or thalline, but the reaction due to these drugs is not affected by heat.

By the distillation of diabetic urine, which gives the above red or violet reaction with ferric chloride, a distillate is obtained containing acetone and alcohol. The products are usually attributed to the decomposition of ethyl aceto-acetate, which would split up with formation of equal molecules of alcohol and

acetone, thus :— $CH_3.CO.CH_2.CO.O(C_2H_5) + H_2O = CH_3.CO.CH_3 + CO_2 + HO(C_2H_5)$. But in a case investigated by Tollens no alcohol appeared in the distillate, although much wine was taken. By treating the distillate with soda and iodised potassium iodide, and weighing the iodoform produced, the acetone in the urine was found to vary from 0·093 to 0·147 per cent. From the production of the iodoform reaction by the distillate, and the absence of alcohol therein, Tollens concluded that the body producing the reaction with ferric chloride was free aceto-acetic acid, and not its ethyl ester.

Tollens further found that urine giving the ferric chloride reaction, when distilled slowly in presence of hydrochloric acid, yielded distillates which gave the iodoform reaction; while the residual liquid, even to the end, continued to show the ferric chloride reaction. The original urine yielded to ether a mere trace of the body giving the violet reaction, but after acidification with sulphuric acid ether extracted it readily.

Hydroxybutyric Acid. $CH._3CH(OH).CH._2COOH$.

The kind of hydroxybutyric acid present in urine, and represented by the foregoing formula, is the β-modification. It was first discovered in the urine by Minkowski, and first obtained synthetically by acting on ethyl aceto-acetate with water and sodium-amalgam. It is also formed when propylene chlorhydrin is heated to 100° with alcohol and potassium cyanide, and the nitril thus produced decomposed by caustic potash. The resultant liquid is acidulated with sulphuric acid and shaken with ether. The ethereal layer, when evaporated, leaves the hydroxybutyric acid as a viscid deliquescent syrup, volatile in a current of steam, but converted, when heated alone to 130° C., into α-crotonic acid, with loss of the elements of water. As prepared

from diabetic urine, by an adaptation of the foregoing process, hydroxybutyric acid has a lævo-rotatory power of $-23{\cdot}4^{\circ}$ for the sodium ray. The *sodium salt* forms very deliquescent needles. The *zinc salt* is amorphous.

Hydroxybutyric acid is poisonous when introduced into the circulation. With ferric chloride it yields a claret or violet-red colour similar to that produced by aceto-acetic ether.

The formation of β-hydroxybutyric acid by the action of nascent hydrogen on aceto-acetic acid has already been referred to; the quantities of the two bodies present in diabetic urine are stated by Wolpe to rise and fall together.

ALBUMINOUS URINE.

NORMAL urine is almost, if not entirely, free from any trace of albumin [1] or other of the allied substances classed together under the generic name of "proteids." [2]

[1] The term "albumen" should be limited to its original signification, namely, the white of egg; the word "albumin" being applied to the most characteristic constituent thereof, and extended to other analogous substances contained in blood-serum, &c. The terms albumen and albumin will then have the same relation to each other as benzol and benzene. In pronouncing the word albumin, it is correct to accentuate the penultimate, but of late years extensive custom has justified the accentuation of the first syllable.

[2] The bodies called proteids are highly complex and, for the most part, uncrystallisable compounds of carbon, hydrogen, nitrogen, oxygen, and sulphur, having an ultimate composition ranging, according to Hoppe-Seyler, within the following limits:—

	Carbon.	Hydrogen.	Oxygen.	Nitrogen.	Sulphur.	
From	51·5	6·9	20·9	15·2	0·3	per cent.
To	54·5	7·3	23·5	17·0	2·0	,,

Proteids occur in a solid viscous state or in solution in nearly all the solids and fluids of the animal economy. The different individuals of the group present differences in physical and, to some extent, in chemical properties; but all possess many chemical reactions in common, and they are united in a close genetic relationship.

W. D. Halliburton (*Watts' Dict. Chem.*, 1894, iv. 330) classifies proteids as follows:—

I. *Albumins.* Soluble in water, and not precipitated by saturating their solutions with common salt or magnesium sulphate. Coagulated by heat. Egg-albumin, serum-albumin, and lact-albumin are the most important members of the group.

II. *Globulins*, represented by serum-globulin, fibrinogen, myosin, crystallin, and globin. They are insoluble in water, but soluble in dilute solutions of neutral salts, and are precipitated in an uncoagulated condition by saturating these solutions with common salt or magnesium sulphate. Globulins are coagulated by heat.

III. *Albuminates*, represented by alkali-albumin, acid-albumin or syntonin, and metallic compounds of proteids. Acid- and alkali-albumin are insoluble in water and solutions of neutral salts, but readily soluble in dilute acids and alkalies. They are not coagulated by heat.

IV. *Proteoses* are proteids which are not coagulable by heat, but are mostly precipitated by certain neutral salts. They are precipitated in the cold by nitric acid, the precipitate dissolving on application of heat, and reappearing

But under particular conditions of fatigue or disease, albumin may appear in the urine.

Temporary albuminuria is sometimes induced by a cold bath, especially in persons prone to kidney-disease, and it has been observed after excessive muscular exercise, as in the urine of soldiers after a prolonged march.

Any cause which leads to increased blood-pressure in the kidneys tends to induce albuminuria, and many of the cases in which it is the result of disease may be traced to this cause. Albuminuria is a constant accompaniment of the nephritis following scarlet fever, and may occur to a less marked extent in pneumonia, typhoid, and diphtheria. It may also occur in diabetes, and is then a highly unfavourable symptom.

The proportion of albumin present in pathological urine varies greatly. In certain diseases the urine sometimes becomes so highly albuminous that on heating it will undergo coagulation in a manner similar to white of egg. More frequently, comparatively small amounts of albumin are found, and in the cases of convalescent patients it is often of importance to ascertain the presence or absence of mere traces of albumin.[1]

as the liquid cools. Proteoses are intermediate in characters between the proteids of Groups I., II., and III. and the peptones. (Compare page 120.)

V. *Peptones* are the ultimate products of the action of proteolytic ferments on the bodies of the preceding groups. They are very soluble in water, not precipitated by neutral salts or nitric acid, and not coagulated by heat. They give the biuret reaction, are incompletely precipitated by picric, phosphotungstic, and phospho-molybdic acids, but completely by tannin, potassio-mercuric iodide, and excess of absolute alcohol. Peptones are divided into *hemipeptones*, which yield leucine and tyrosine as the further result of pancreatic digestion, and *antipeptones* which do not give these products.

VI. *Insoluble proteids.* This class includes a number of bodies not classified above, but which resemble each other in their extreme insolubility in various reagents. Among them are fibrin, coagulated albumin and casein, lardacein, antialbumid, and gluten.

[1] In cases of Bright's disease, the urine rarely contains more than 1 per cent. of proteids; but taking the volume of urine at the moderate estimate of 50 oz. daily, this corresponds to a loss of about 220 grains, or 14 grammes per diem. Freund has pointed out that the blood of the average body contains only about 450 grammes of albumin, so that if only 7 to 8 grammes of this be lost daily the condition is alarming.

	Serum Albumin.	Serum Globulin.	Hetero-Proteose.	Deutero-Proteose.	Peptones.	Acid-Albumin.	Alkali-Albumin.	Mucin.
Dilution with water, .	No change.	Slight opacity	No change.	No change.	No change.	No change.	No change.	No change.
Saturation with magnesium sulphate (page 118),	No change.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	...
Saturation with ammonium sulphate (page 122),	Precipitate.	Precipitate.	Precipitate.	Precipitate.	No change.	Precipitate.	Precipitate.	...
Reaction on boiling after slight acidulation with acetic acid (page 105),	Precipitate.	Precipitate.	No change.	No change.	No change.	No change.	Precipitate.	No change.
Cold concentrated nitric acid (page 107),	Precipitate.	Precipitate.	Precipitate, soluble in excess or on heating, reappearing on cooling.	Precipitated only on adding brine, dissolves on heating, reappears on cooling.	No change.	Precipitate.	Precipitate.	Precipitate.
Metaphosphoric acid (page 113),	Precipitate.	Precipitate.	...	...	...	No change.	Precipitate.	...
Picric acid (page 110), .	Precipitate.	Precipitate.	...	Precipitate, soluble on heating.	Precipitate, soluble on heating.	Precipitate.	Precipitate.	No precipitate unless acetic acid be added.
Potassium ferrocyanide (page 108),	Precipitate.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	...
Potassio-mercuric iodide (page 114),	Precipitate.	Precipitate.	...	Precipitate, soluble on heating.	Precipitate, soluble on heating.	Precipitate.	Precipitate.	Precipitate.
Fehling's solution (biuret reaction) (page 123),	Brown-red or violet.	Brown-red or violet.	Rose-pink.	Rose-pink.	Rose-pink.	Brown-red or violet.	Brown-red or violet.	Violet.
Cupric sulphate, . . .	Precipitate.	Precipitate.	Precipitate.	No change.	No change.	Precipitate.	Precipitate.	...

Detection of Albumin in Urine.

Until recently, the proteids liable to occur in urine were classed together under the general name of "albumin," but it is now recognised that several forms are of common and simultaneous occurrence, and apparently have a varied pathological significance.

The table on page 103 exhibits in a convenient form the chief reactions of solutions of the proteids which are liable to occur in urine.

For clinical use and medical purposes generally, it is necessary to employ simple but fairly delicate tests for the detection of albumin in urine, and many attempts have been made to fulfil the requisite conditions. The following are among those tests which experience has shown to be most generally available and reliable for the purpose, but the recognition of mere traces of albuminous matters in urine is often of great importance, and to effect this with certainty the tests must be applied with care and skill.

Urine to be examined for albumin should, by preference, be the mixed excretion of the previous twenty-four hours; but it is easy to lay too much stress on this desideratum. It is better to have a carefully collected sample of the urine passed at one time than a sample of mixed urine collected under conditions open to exception. Thus, in collecting a sample of urine to be examined for albumin, it is important to reject the first ounce or two passed, in order to wash casual discharges out of the urethra. The urine which follows should be passed direct into the sample-bottle, which must be scrupulously clean and may conveniently contain six ounces.

Before applying any of the following tests for albumin *it is essential that the urine to be examined should be filtered*, so as to obtain an absolutely clear liquid, and to ensure its freedom from casual contami-

nation with semen, mucus, epithelial cells, or other debris from the urinary passages.[1]

Previous to filtration, it is important to observe the reaction of the urine. If a slip of blue litmus-paper be promptly reddened when dipped for an instant into the urine, the liquid may at once be filtered. Urine passed during the so-called alkaline tide (page 9) may fail to redden blue litmus, or may even restore the blue colour to reddened litmus-paper. In such case, the urine, before filtration, should be acidulated by adding dilute acetic acid,[2] drop by drop, with frequent agitation to ensure perfect homogeneity, until the attainment of a proper degree of acidity is marked by the prompt reddening of a slip of immersed litmus-paper. The urine thus treated will, on filtration, yield a perfectly bright filtrate, which practically is the true urinary excretion, will give no trouble from the presence of mucin, and will yield no precipitate of earthy phosphates or other salts on boiling.

Heat Test.—One of the simplest, and in many cases most satisfactory, tests for the presence of albumin in urine is that of heat. About 10 c.c. (or ½ oz.) of the sample, previously filtered and, if necessary, acidulated, as above described, is boiled for about one minute in a test-tube. If albumin be present, as the boiling point is approached a cloud is seen to form at the top of the liquid, and as the urine is boiled the whole of the albumin separates as a soft, white, opaque precipitate, more or less dense according to the

[1] To ensure perfect filtration, which, when practicable, should be performed on the fresh, warm excretion, a fine close filter-paper should be employed. The importance of operating on carefully filtered urine has been pointed out and insisted on by James Edmunds (*Lancet*, November 9th, 1889, page 978).

[2] The use of too strong an acid should be avoided. *Dilute acetic acid* of the Pharmacopœia contains 4·27 per cent. of real acetic acid, and is a suitable reagent for the purpose; or *normal acetic acid* containing 6 per cent. of real acid, which can be obtained approximately by diluting 2 fluid ounces of *Acetic Acid*, B.P., with 11 fluid ounces of distilled water, may be employed.

proportion which may be present. On standing for a few minutes this precipitate aggregates into distinct flocculi, and these gradually sink to the bottom of the test-tube, leaving the supernatant liquid clear. After standing for twenty-four hours, the volume occupied by the precipitate will afford a rough indication of the proportion of albumin present. (Compare Esbach's test, page 111.) In highly albuminous urines the precipitate is occasionally so voluminous as to cause the coagulation of the entire liquid.

By the foregoing simple mode of procedure, any proportion of albumin greater than traces will be readily detected. For the detection of smaller quantities, equal measures of the carefully filtered acidulous urine should be placed in two exactly similar test-tubes. The liquid in one tube is then boiled, when, on comparing its appearance with that in the other tube, placed side by side with it, the faintest opalescence will be readily perceived, especially if the tubes be observed in a proper light, with a black background for the line of vision. An alternative plan is to boil the upper part of a column of urine in a somewhat long test-tube by means of a small flame. Any albumin in the upper heated portion of the liquid will thus be coagulated, and will present a marked contrast to the pellucid lower layer.

If not in a distinctly acidulous condition, human urine, on boiling, often yields a precipitate of earthy phosphates, while calcium carbonate is sometimes thrown down from the urine of herbivorous animals. These precipitates are readily and completely re-dissolved on adding a few drops of acetic acid, with agitation between each addition, while a precipitate of albumin will remain unchanged under such treatment. A pulverulent precipitate of earthy phosphates, usually $CaHPO_4$, is easily distinguished from the fine

flocculi into which an albuminous precipitate soon aggregates. From alkaline urine, albumin is not thrown down by boiling.

In conducting the heat test for albumin, one of the essentials of success is to have the liquid acidulated, as already stated, to a suitable extent. The urine should sharply redden blue litmus-paper, but excess of acid must be avoided. Occasionally it is desirable to make several tests on portions of the sample to which gradually increased quantities of acetic acid have been respectively added.

According to Tyson, the addition of a few drops of acetic acid may diminish an albuminous precipitate, but on adding more re-precipitation occurs. A large excess, especially if the liquid be boiled, will permanently dissolve the precipitate of albumin. Nitric acid is preferred to acetic acid by some operators, but even more care is necessary to avoid the use of an excess, and the reagent is unsuited for the study or bedside.

C. W. Purdy (*Practical Uranalysis*, 1894) recommends that the urine should be treated with sufficient of a saturated filtered solution of common salt to raise the gravity to about 1035. One or two drops of acetic acid should then be added and the upper portion of the liquid boiled, as already described. The addition of the brine is stated to prevent any precipitation of mucin, and hence to avoid the confusion thereby occasioned. But Purdy, apparently, omits to filter the acidulous urine, which would practically remove the mucin.

Nitric Acid Test.—Another delicate and simple test for albumin in urine is due to Heller, and is based on its coagulation by cold nitric acid. The simple addition of some of the urine to strong nitric acid contained in a test-tube, in such a manner as to prevent the liquids from mixing, suffices for the detection of notable quantities of albumin, but the

reaction is much increased in delicacy and reliability by operating in the following manner, devised by Sir William Roberts. A saturated solution of magnesium sulphate is prepared by dissolving 10 parts of the crystallised salt in 13 of hot water and filtering the liquid. To 5 measures of this solution, 1 of nitric acid of 1·42 specific gravity is added. This reagent is so dense that it is easy to avoid admixture with the urine to be tested. Some of the acid mixture is placed in a test-tube, and an equal or larger measure of the filtered urine allowed to flow gently on to the surface, carefully avoiding any mixing of the two layers.[1] In presence of much albumin a more or less opalescent zone is immediately formed at the junction of the two liquids; but when only traces are present a longer time, sometimes extending to a quarter of an hour, is requisite for the development of the band. The turbidity due to albumin occurs at the bottom of the layer of urine, just above the line of demarcation, while any cloudiness due to mucin[2] always appears as a diffused haze towards the upper part of the liquid, and therefore quite distinct from the albumin ring. A stratum of uric acid occasionally separates in applying this test, but it disappears on warming. With some urines a crystalline precipitate of nitrate of urea may form, but this cannot be mistaken for albumin.

Ferrocyanide Test.—Another very delicate test for albumin is to treat the suspected urine with excess of acetic acid, and then add an aqueous solution of

[1] J. E. Saul (*Pharm. Jour.*, [3], xvii. 857) suggests the use of a small glass syringe instead of a test-tube. About an inch of the clear urine is first drawn up into the syringe, and then a sufficiency of the reagent. In this manner the danger of inadvertently mixing the two layers is much lessened.

[2] After taking copaiba balsam or sandal oil, the urine may contain resin acids, precipitable by nitric acid, and therefore liable to be mistaken for albumin. In such cases, Alexander recommends that 10 c.c. of the urine should be treated with two or three drops of hydrochloric acid, which will precipitate the resin acids. If, on adding acetic acid, a precipitate is formed, insoluble in excess of the reagent, this consists of mucin.

potassium ferrocyanide. A white precipitate will form immediately, or after a short interval, if any trace of albumin be present.[1] The reaction is delicate, and produced only by coagulable proteids, which is important, since peptones may be present in urine without albumin. If true albumin be present, there will be paraglobulin and myosin as well. This is stated to occur in amyloid degeneration of the kidney.

F. W. Pavy employs tabloids containing citric acid and potassium ferrocyanide, instead of using solutions of the reagents.[2] For clinical purposes, this plan is very convenient.

G. Oliver, of Harrogate, has proposed to employ strips of filter-paper impregnated with the reagents. By immersing one of the potassium ferrocyanide papers and another of the citric acid papers in a little (5 c.c.) distilled water, the reagent can be prepared in a few minutes.

C. W. Purdy (*Practical Uranalysis*) insists strongly on the importance of adding the acetic acid and ferrocyanide solution simultaneously, or at any rate the latter reagent before the acid. In this way he states that any precipitation of mucin is wholly avoided. To a test-tube half filled with the sample of

[1] A yellow coloration is sometimes produced on adding the ferrocyanide solution to urine. According to J. P. Karplus (*Chem. Centr.*, 1893, ii. 496) this reaction is due to nitrites, which he states are often present in urine which has been kept more than twenty-four hours, but not in the fresh excretion.

[2] Pavy directs that a pellet of citric acid should be dropped into about a drachm (= 3·5 c.c.) of the urine, and the liquid agitated till solution occurs. A ferrocyanide pellet is then added, and the fluid again agitated. An immediate precipitate occurs if albumin be present. If the urine be turbid from the presence of urates, it must be previously filtered or clarified by heating, but otherwise heating is unnecessary. A turbidity on adding the citric acid only may be due to either uric acid or mucin. In the former case, previous dilution of the sample with an equal measure of water prevents its formation; in the latter, the density of the precipitate produced after adding the ferrocyanide should be compared with that produced by citric acid alone. The ferrocyanide test is less delicate when citric acid is employed than when acetic acid is used, but Pavy's modification is very useful for clinical purposes. The pellets may be obtained from Mr Cooper, 66 Oxford Street, London.

urine, Purdy adds "a drachm or so" of a 5 per cent. solution of potassium ferrocyanide, and after agitating adds from 10 to 15 drops of acetic acid. No reaction is said to be produced by mucin, peptones, urates, phosphates, alkaloids, or resin acids. Any precipitate is due to albumin and nothing but albumin.

Zouchlos proposes to substitute *potassium thiocyanate* (sulphocyanide) for the ferrocyanide. He prepares the reagent by mixing 10 c.c. of a 10 per cent. solution of the salt in water with 2 c.c. of acetic acid.

A. Ollendorff (*Zeitschr. anal. Chem.*, xxxiii. 120) confirms the value of Zouchlos' test, and states that it is capable of detecting 0·005 per cent. of albumin, while other constituents of urine, with the exception of propeptone, have no disturbing influence.

Picric Acid Test.—A valuable reagent for the detection of albumin in urine is a cold saturated aqueous solution of picric acid, first proposed by Braun, and since strongly advocated by Sir G. Johnson and others. (*Brit. Med. Jour.*, Oct. 11, 1884; *Analyst*, ix. 206.) This may be added either to the original (filtered) urine, or to a portion in which acetic acid has failed to give a precipitate, and either the cold or hot urine may be employed. The reagent may either be allowed to mix with the urine, or the junction of the two layers may be observed, when a turbidity will be produced if any trace of albumin be present. For clinical purposes, a minute quantity of powdered picric acid may be substituted for the solution of the reagent. Peptones, alkaloids, piperazine, and urates (when in large excess) are liable to give precipitates with picric acid; but these precipitates are readily distinguished from that due to albumin by their disappearance on heating the liquid. In the absence of acetic or other added acid, picric

acid does not precipitate mucin, and this fact forms a valuable distinction between that body (said by Sir G. Johnson to be always present in traces even in normal urine) and true albumin.

Esbach's Test.—A very fair approximation to the quantity of albumin contained in urine is said to be obtainable in the manner devised by Esbach, who performs the test in a graduated tube, about 6 inches in length and 0·6 inch in diameter. Esbach's reagent is prepared by dissolving 10 grammes of picric acid and 20 of citric acid in about 900 c.c. of boiling water, and after cooling making up the volume to 1 litre by adding water. (Or ¼ oz. of picric and ½ oz. of citric acid may be dissolved in 25 oz. of water.) The tube is filled to the mark "U" with the urine to be tested, and the reagent added up to the mark "R." The liquids are mixed by cautiously inverting the closed tube several times, avoiding strong agitation, and then left at rest for twenty-four hours. The volume of the precipitate is then observed, each degree corresponding to 0·1 per cent. of albumin.[1] The process is inapplicable to urine containing less albumin than 0·1 per cent., and samples containing more than 0·7 per cent. must be previously diluted with their own or twice their measure of water, as the tubes are not graduated for more highly albuminous urines.

Fig. 6.—Esbach's Tube.

[1] The graduation of the tubes is purely empirical. The results are commonly declared to be quite accurate enough for all clinical requirements. In the experience of the author, the results are fairly comparable if the same conditions as to time, &c., are observed, but the absolute quantities of albumin indicated leave much to be desired in point of accuracy, and wide discrepancies occur if the reading be taken much before or after the twenty-four hours prescribed.

Esbach's tubes are obtainable from E. Cetti, 36 Brooke Street, Holborn, London; A. Gallenkamp & Co., Cross Street, Finsbury, London; Southall & Son, Birmingham; or Gibbs, Cuxson, & Co., Wednesbury.

A greatly improved form of Esbach's tube has been devised by C. W. Purdy, of Chicago.[1] The tube is drawn out into a blunt cone at the closed end, so that much smaller quantities of albumin can be measured. Dr Purdy makes the further great improvement of placing the tube in a centrifugal machine (such as the Leffmann-Beam apparatus for estimating fat in milk), whereby complete separation of the albumin can be effected in a few minutes, instead of after standing twenty-four hours.

TRICHLORACETIC ACID, $CCl_3.COOH$, is a reagent for albumin proposed by Raabe, and a test for which special advantages are claimed. The reagent does not precipitate peptones nor coagulate mucin. It is said to precipitate a form of albumin not indicated by either of the previously described tests, which form appears to be specially characteristic of the presence of granular. epithelial, or hyaline casts. Trichloracetic acid used in saturated solution, and poured on the cold urine, will detect 1 part of albumin in 100,000 of liquid. In presence of quinine or other alkaloids a small addition produces a dense white precipitate, soluble on heating or on adding a large excess of the acid.

SALICYL-SULPHONIC ACID[2] is recommended by M. Roch (*Archiv. des Pharm.*, xxvii. 998) and J. A. MacWilliam (*Brit. Med. Jour.*, i., 1891, page 837) as a precipitant of all varieties of proteids in urine. In applying the test it is simply necessary to add a few crystals of the reagent to a small quantity of the clear urine and agitate, when the appearance of a turbidity or an actual precipitate will indicate the presence of albumin. The precipitate produced

[1] Obtainable from Messrs Eimer & Amend, 205 Third Avenue, New York.

[2] Salicyl-sulphonic acid or sulpho-salicylic acid is prepared by heating salicylic acid with twice its weight of sulphuric anhydride at 100° C. until dissolved. On cooling and standing brownish crystals of the compound separate. These are purified by recrystallisation from boiling water.

by albumins and globulins is not affected by heat, while that due to albumoses and peptones dissolves, reappearing as the liquid cools. No normal or abnormal constituent of urine other than proteids is precipitated by the reagent, while these are very completely separated. One part of egg-albumin in 20,000 of water can be detected.

METAPHOSPHORIC ACID, readily obtained by dissolving glacial phosphoric acid in cold water, is recommended by C. Hindenlang (*Chem. Centralb.*, 1881, page 471) for the detection of albumin in urine. To obviate the inconvenience caused by the ready conversion of the meta- into ortho-phosphoric acid, L. Blum (*Chem. Centralb.*, 1887, page 345) recommends the following reagent:—From 0·03 to 0·05 gramme of manganous chloride is dissolved in a little water, acidulated with a few c.c. of dilute hydrochloric acid, and treated with 100 c.c. of a 10 per cent. solution of sodium metaphosphate (best prepared by igniting microcosmic salt in platinum). Lead dioxide is then added, in small quantities at a time, with constant agitation, the liquid is allowed to settle, and filtered. The resulting pink solution of manganic metaphosphate is used as an indicator of albumin. The reagent should be placed in a test-tube and the urine to be examined filtered into it. The solution is said to keep well, and to give no reaction with other constituents of urine.

SPIEGLER'S TEST (*Ber.*, xxv. 375) consists of a solution of 8 grammes of mercuric chloride, 4 of tartaric acid, and 20 of sugar, in 200 c.c. of water. On running onto the surface of this reagent some of the urine (previously acidulated with a little strong acetic acid and filtered if necessary), a distinct white ring is formed at the line of demarcation if albumin be present. Globulin and hemi-albumose behave simi-

larly, but peptones give no reaction. The addition of sugar to the reagent is simply for the purpose of increasing the density, and so avoiding mixture with the stratum of urine.

POTASSIO-MERCURIC IODIDE has been recommended by Tanret as a precipitant of albumin.[1] The reagent is prepared by dissolving 1·35 gramme of mercuric chloride and 3·32 of potassium iodide in 64 c.c. of water, and adding 20 c.c. of acetic acid. Méhu states that this reagent precipitates mucin. Brasse found peptones and alkaloids to be thrown down, but the precipitates due to these bodies dissolve on heating, leaving that due to albumin. The alkaloidal precipitate is soluble in ether, the peptone precipitate insoluble. Bile-salts gave a precipitate not dissolved by heat, but distinguishable from that due to albumin by its solubility in ether.

No reaction with Tanret's reagent is produced by creatine, creatinine, xanthine or hypoxanthine.

Various other reagents have been proposed for the

[1] Tanret has proposed to determine the albumin volumetrically by acidulating the urine with acetic acid and adding a standard solution of potassio-mercuric iodide until a drop of the liquid yields a precipitate on addition of mercuric chloride. The method is said to give fair clinical results.

F. Venturini suggests a modification of Tanret's process based on the fact that mercuric chloride precipitates albumin from urine acidulated with acetic acid before reacting with potassium iodide. A standard solution of mercuric chloride, containing 10 grammes per litre, is prepared. Each 1 c.c. of this solution precipitates 0·0245 gramme of albumin. To 5 c.c. of the urine, 6 c.c. of a 5 per cent. solution of potassium iodide is added, together with a few drops of acetic acid, and the standard mercuric chloride then added drop by drop, until a permanent yellowish-red coloration is obtained. From the volume used a deduction of 1 c.c. is made for the excess required to show the coloration, and the difference is multiplied by the factor 0·0245 to obtain the amount of albumin.

Georges (*Jour. Pharm.*, [5], xiii. 353) utilises these facts for the detection of peptones in the following manner. The coagulable albumin is first precipitated by heating the urine. The filtrate is precipitated by Tanret's reagent, and the precipitate washed on the filter with cold water charged with acetic acid to the same extent as the urine. It is then washed with the same acidulated water heated to boiling, the washings being kept separate. The clear liquid thus obtained gives a precipitate on cooling if any trace of peptone has been dissolved.

detection of albumin in urine, but their behaviour with co-occurring bodies has either been incompletely studied or they present no tangible advantages over the tests already described.

Grainger Stewart (*Edin. Med. Jour.*, May 1887) is of opinion that picric acid is the most delicate of all reagents for albumin, Tanret's reagent ranking second. U. Vetlesen, of Christiania, represents the relative delicacy of the various tests by the following figures:—Nitric acid, 85; trichloracetic acid, 82; potassium ferrocyanide and acetic acid, 82; metaphosphoric acid, 72; picric acid in solution, 36; sodium sulphate and acetic acid, 25. D. Campbell Black (*Urine and Urinary Analysis*), as the result of careful experiments, considers Tanret's reagent the most delicate; heat, nitric acid, and the aceto-picric solution following in the order named; and ferrocyanide being one of the least delicate of the tests tried.[1]

[1] Recent experiments by E. J. Evans (*Pharm. Jour.*, [3], xxv. 913), with a view of comparing the relative delicacy of different reagents, gave the following results with solutions of egg-albumin. Solution No. 1 had a strength of 1 in 40; solution No. 2 of 1 in 200; while solution No. 3 contained 1 part of albumin in 1000 of water. Half an ounce (8 c.c.) was employed for each experiment. No indication is given in the paper whether dry albumin or fresh egg-albumen was employed.

On boiling, No. 1 showed coagulation and slight opalescence, but only frothing was observed in the case of solutions 2 and 3.

On adding a few drops of acetic acid and heating, No. 1 coagulated; No. 2 gave a white froth with slight coagulation; while No. 3 showed a slight froth, without any sign of coagulation or opalescence.

Heated with a few drops of nitric acid, solution 1 gave a cloudy precipitate, which became denser on heating; No. 2, a white cloud in the line of the drops, and on shaking a white opalescence; while No. 3 showed only a slight froth without opalescence.

Picric acid gave with No. 1 a bulky yellow precipitate, soluble in excess of ammonia; with No. 2 a yellow opalescence, and after heating and standing for some time a yellowish precipitate; and No. 3 behaved somewhat similarly.

With a nitric acid solution of ammonium molybdate No. 1 gave a white precipitate, separating in flocks when heated; No. 2 a white opalescence; and No. 3 the same.

Uranium acetate produced with No. 1 a yellowish-white precipitate, curdling on heating; with No. 2 a yellowish-white opalescence, a precipitate separating on heating; and much the same with No. 3.

Determination of Albumin in Urine.

The method of Esbach (page 111) affords quantitative results which are somewhat rough but sufficiently accurate for many purposes. More exact determinations can be obtained by precipitating the albumin, &c., by a suitable reagent, and drying and weighing the washed precipitate, or estimating the nitrogen contained in it.[1] From the nitrogen found, the proteids are calculated by multiplying by the factor 6·3.[2] Some observers employ the factor 6·25, and others as high a factor as 6·37. 6·3 is a fair average figure, and sufficiently accurate for all purposes. Picric acid and ferrocyanide of potassium are inapplicable as precipitants, since they contain nitrogen.[3] Tannin, carbolic acid, or trichloracetic acid may be used; or Tanret's solution of potassio-mercuric iodide may be employed, bearing in mind the substances other than albumin which are liable to be thrown down. Of the available precipitants, carbolic acid and tannin appear to be the best.

The following method of determining albumin in urine is strongly recommended by Méhu. 100 c.c. measure of the cold urine is rendered slightly acid with acetic acid, 2 c.c. of concentrated nitric acid added, and the liquid thoroughly agitated. Ten c.c. of a mixture of 1 part by weight of crystallised carbolic acid, 1 of commercial acetic acid, and 2 of rectified spirit is next added, the liquid mixed thoroughly, and filtered after a few minutes. The filtration proceeds rapidly. The precipitate is washed with a cold 4 per

[1] Kjeldahl's process is well-adapted for this purpose. The modification of it described on page 128 is convenient when approximate results will suffice.

[2] The employment of this factor is based on the composition of albumin and its allies, which contain on the average 15·88 per cent. of nitrogen; and $100 \div 15{\cdot}88 = 6{\cdot}3$.

[3] The nature of the precipitates produced by these reagents in solutions of albumin appears to be uncertain. According to some writers at least, the precipitates are actual compounds of the reagent with the proteid.

cent. solution of carbolic acid in water,[1] when it may either be dried and weighed, or treated (paper and all) by the modified Kjeldahl's method, and the nitrogen found multiplied by 6·3 to obtain the weight of the proteids precipitated. The presence of sugar or much saline matter in no way affects the accuracy of this process, but in the presence of a large proportion of salts the addition of nitric acid becomes unnecessary.

Van Nuys and Lyons (*Amer. Chem. Jour.*, xii. 336; *Analyst*, xv. 234; xvi. 7) have described a method of determining albumin dependent on its precipitation by a solution of tannin. This is prepared, according to the method of Almén, by mixing 4 grammes of tannic acid, 8 c.c. of acetic acid (1 part of glacial acid to 3 parts of water), and 190 c.c. of 50 per cent. alcohol. Ten c.c. of this solution and an equal measure of the filtered urine are mixed well, and the liquid passed through a dry filter. In 5 c.c. of the filtrate the nitrogen is then determined by Kjeldahl's process, and by the same method the nitrogen is determined in 5 c.c. of the original urine. The difference between the two results represents the nitrogen precipitated by the tannin reagent, and this amount multiplied by 6·3 gives the corresponding weight of albumin and globulin in the precipitate. If the urine contain more than 2 per cent. of albuminoids it should be diluted with an equal or double measure of water, and the calculation modified accordingly.

H. O. G. Ellinger (*Jour. Prakt. Chem.*, [2], xliv. 256) has described a method of determining albumin in urine by means of an instrument closely allied to Amagat and Jean's refractometer. The sample in its

[1] According to Méhu's original directions, the precipitate is to be washed with boiling water containing 1 per cent. of carbolic acid. L. Ruisand (*Jour. Pharm.*, [5], xxix. 364) finds that a very appreciable amount of albumin is dissolved by this treatment, but that no appreciable solution occurs when the washing is conducted as described in the text.

original state is compared with the same urine from which the albumin has been removed by heat and acetic acid. The results agree somewhat roughly with the gravimetric determination.

Distinction and Separation of Urinary Proteids.

Until recently it was not recognised that more than one proteid body was liable to occur in urine, but it is now known that serum-globulin (paraglobulin) frequently co-exists with serum-albumin, and, according to Senator, invariably accompanies the latter substance or even exists alone. Certain albumoses, proteoses or propeptones may also be present, in addition to which true peptones sometimes occur either with or without serum-albumin. Traces of mucin are usually present, and fibrin and hæmoglobin may occur in certain septic and purpurous conditions.

PARAGLOBULIN may be detected in urine, if present in large amount, by diluting the liquid with two measures of distilled water, rendering it faintly acid, and passing a stream of carbon dioxide. On standing for twenty-four hours or more, the globulin forms a white flocculent precipitate.

Noel Paton (*Brit. Med. Jour.*, 1890, page 197) has described the following method of separating and estimating paraglobulin and serum-albumin when occurring together in urine. The total proteids present are first determined by Esbach's method. Fifty c.c. measure of the urine is then rendered faintly alkaline, and powdered magnesium sulphate added until the liquid is saturated. It is then allowed to stand in a warm place for twenty-four hours, when the globulin will be completely precipitated. The liquid is then measured, filtered, and a portion again treated by Esbach's method (page 111). The result now obtained, after due allowance for the increased volume,

represents the albumin of the urine, and the difference between this and the figure previously obtained will be the globulin (*plus* any hemi-albumose) of the urine. The globulin and hemi-albumose ("heteroproteose") may be separated, if desired, by redissolving the precipitate produced by ammonium sulphate in a small quantity of water, and treating the solution obtained with ten times its measure of absolute alcohol. The resultant precipitate is collected and digested with cold absolute alcohol for a week or ten days. The liquid is then filtered, when a residue will consist of globulin, and the hemi-albumose will be found in the filtrate.

In the urine of a person recovering from a prolonged attack of diarrhœa, Paton found 2 per cent. of total proteids, of which 1·92 was globulin; and in another case 3·82 of total proteids, of which 3·73 was globulin, which was obtained in elongated rhombic crystals.

Instead of employing Esbach's method, A. Ost (*Chem. Centralb.*, 1884, page 500) observes the optical activity of the urine before and after saturation with magnesium sulphate.

An alternative plan is to precipitate the globulin and albumin together by boiling the faintly acidulated urine, and estimate the nitrogen in the washed precipitate by the author's modification of Kjeldahl's method (page 128). In another portion of the urine the globulin is precipitated by saturating the liquid with magnesium sulphate, the precipitate collected and washed with magnesium sulphate solution, and the contained nitrogen estimated by the modified Kjeldahl's process. The difference between the two results is the nitrogen corresponding to the albumin of the urine.

According to Senator, paraglobulin occurs in urine in cases of lardaceous disease of the kidneys, and has also been found in excess in the intense hyperæmia

resulting from poisoning by cantharides, and in functional albuminuria associated with marked disturbance of the digestive organs. The greater the proportion of paraglobulin present, the more unfavourable appears the diagnosis in Bright's disease. When blood is present in urine, as in nephritis after scarlet fever, there is a large increase in the proportion of paraglobulin. Noel Paton (*Brit. Med. Jour.*, ii., 1890, page 196) finds the ratio of globulin to albumin to vary enormously (from 1 : 0·6 to 1 : 39). It varies much during the day, and in such an erratic manner that no conclusions can be drawn.

Albumoses or Proteoses.

Of late years a series of bodies, intermediate in characters and composition between the albumins and peptones, have been isolated, and some of these have been proved to exist in certain forms of pathological urine. Thus Gerrard has recently shown (*Pharm. Jour.*, [3], xxiii. 261) that in the milk-treatment of albuminuria no albumin coagulable by heat exists in the urine, but that nitric acid gives a precipitate soluble in excess, or on warming, reappearing on cooling; and saturated brine a flocculent precipitate increased by the addition of acetic acid. These reactions are characteristic of the form of proteose called hetero-albumose or hetero-proteose, the presence of which in urine is said to be an indication of approaching nephritis. The same substance has been found in cases of osteo-malacia and atrophy of the kidneys, and in the urine of persons who have been rubbed with petroleum.

For the detection of hetero-albumose, Tyson acidifies the urine with a few drops of acetic acid, and adds one-sixth of its volume of saturated brine, boils, and filters. Albumin and globulin are precipitated. If the filtrate after cooling gives a precipitate on

further addition of brine, which dissolves on heating and reappears on cooling, the presence of albumose is indicated.

For the distinction of the proteoses liable to occur in urine, and their separation from co-occurring proteids, the following plan may be used. It is taken, with slight modifications, from W. D. Halliburton's *Text-book of Chemical Physiology and Pathology.*

<table>
<tr><td colspan="4">Bring the urine to a faintly acid condition by cautious addition of acetic acid or dilute caustic alkali (as the original reaction to litmus may indicate), boil for a minute or so, and filter.</td></tr>
<tr><td rowspan="4">PRECIPITATE consists of *albumin* and *globulin*. These may be separated by saturating another portion of the faintly acidulated urine with powdered magnesium sulphate, which precipitates *globulin*, *proto-albumose*, and *hetero-albumose*. From the filtrate, albumin will be precipitated on boiling, while deutero-albumose and peptones remain in solution.</td><td colspan="3">FILTRATE, allow to cool, saturate thoroughly with powdered ammonium sulphate, and filter.</td></tr>
<tr><td colspan="2">PRECIPITATE consists of albumoses. Wash with a saturated aqueous solution of ammonium sulphate. Redissolve the washed precipitate in a minimum quantity of water. Faintly acidulate the solution with acetic acid, saturate it with common salt, and filter.</td><td>FILTRATE contains *peptones* only, recognisable by xanthoproteic and biuret reactions, as on page 123.</td></tr>
<tr><td>PRECIPITATE consists of *primary albumoses*. Wash with saturated brine, redissolve by adding water, and dialyse the solution.</td><td rowspan="2">FILTRATE may contain *deutero-albumose*, precipitated by saturating the solution with ammonium sulphate, and recognisable by other reactions on page 123.</td><td rowspan="2"></td></tr>
<tr><td>PRECIPITATE consists of *hetero-albumose*, which may be further identified by reactions on page 120.</td><td>FILTRATE may contain *proto-albumose*, precipitable by excess of alcohol (10 : 1).</td></tr>
</table>

Traces of albumoses are liable to be formed by the action of the boiling acidulated liquid on the albumin and globulin. A sharper separation can be effected by treating the urine with ten times its volume of

strong alcohol, which precipitates all the proteids. The precipitate is rinsed off the filter with absolute alcohol, and left in contact with the alcohol for five to ten weeks. This treatment coagulates the albumin and globulin without affecting the albumoses or peptones. The supernatant alcohol is poured off, the remainder evaporated at a temperature not exceeding 40° C. (= 104° F.), and the residue treated with water, which dissolves the albumoses and peptones, leaving the albumin and globulin insoluble.

PEPTONES are now known to occur in the urine under a great variety of pathological conditions, especially in acute febrile diseases and nervous complaints, and they probably exist in traces in normal urine. The only reliable method of distinguishing and separating peptones from co-occurring proteids appears to be that of S. H. C. Martin (*Brit. Med. Jour.*, i., 1888, page 842). This consists in saturating the urine, faintly acidulated with acetic acid, with ammonium sulphate. The powdered salt is added gradually to the urine till no more is taken up. The precipitate of proteids rises to the surface of the liquid and can readily be separated by filtration.[1] By this treatment any albumin, globulin, or proteose is completely precipitated, whatever the reaction of the liquid, while any peptone remains in solution.

Peptones present the closest similarity in their pro-

[1] If the precipitate be washed twice with a cold saturated solution of ammonium sulphate, and then redissolved on the filter in distilled water, a solution is obtained in which the proteids are easily differentiated. Thus, on saturating the liquid with magnesium sulphate, the globulin is precipitated, while the albumin can be detected in the filtered liquid by its property of coagulating when heated to 73° C., after slightly acidulating the solution with acetic acid. Hemi-albumose is precipitated at 43° to 50° C., the precipitate being soluble in a few drops of a weak acid, and it is precipitated in the absence of acids, which albumin and globulin are not. It also gives a pink reaction with the biuret test, and with nitric acid a precipitate which dissolves on heating and reappears on cooling. It is likewise precipitated from a solution faintly acidulated with acetic acid by potassium ferrocyanide, and by saturating its solution with magnesium sulphate.

perties and reactions to the proteoses, and especially to the body called deutero-proteose or deutero-albumose. Peptones differ from deutero-proteose in not being precipitated on saturating the solution with ammonium sulphate, and in giving no precipitate with nitric acid under any conditions. Deutero-proteose, on the other hand, is precipitated by nitric acid after a considerable quantity of common salt has been added to the liquid. This precipitate dissolves on heating the liquid containing it, but reappears on cooling.

Both peptone and deutero-albumose are precipitated, but not coagulated, by alcohol. They are not precipitated by boiling, nor by cupric sulphate, but are precipitated by phospho-molybdic, phospho-tungstic, picric acid, tannin, or potassio-mercuric iodide. They resemble other proteids in yielding a yellow coloration on boiling with nitric acid, becoming brownish on adding excess of ammonia (the "xantho-proteic reaction"); and by the pink or rose-red coloration obtained on adding excess of caustic alkali, followed by a few drops of a very dilute solution of cupric sulphate ("biuret reaction").

These two reactions can be employed for the detection of peptones in the filtrate from the precipitate produced by saturating the urine with ammonium sulphate, as above described.

Where mucin and albumin are already absent, it is stated that peptones may be detected by treating 50 c.c. of the original urine with 5 c.c. of hydrochloric acid and precipitating the warm liquid with sodium phospho-tungstate. The supernatant liquid is decanted, and the resinous precipitate washed twice with water containing 0·5 c.c. of caustic soda solution of 1·16 specific gravity, which dissolves it. The resultant solution is warmed till a greenish turbidity

is produced, allowed to cool, and a 1 per cent. solution of cupric sulphate added drop by drop. In presence of a peptone, a red coloration is produced, which is rendered more evident by filtering the liquid.

Roux (*Jour. Pharm.*, [5], xxv. 544) proposes to determine the peptones in urine volumetrically. The sample is freed from albumin and reducing compounds, and a decinormal Fehling's solution added until the colour changes through light blue, blue-violet, lilac, and rose-purple, to a greyish tint. One c.c. is said to represent 0·004 gramme of peptone.

MUCIN is the chief constituent of the mucus derived from the renal and urinary passages, and is probably the source of the so-called "animal gum" found in the urine by Landwehr. It occurs very commonly (according to Sir G. Johnson, invariably) in traces even in normal urine, and in larger amount in urinary catarrh and other affections of the urinary organs. Mucin is slightly soluble in neutral or alkaline urine, but is precipitated on adding acetic acid, and is insoluble in excess of the precipitant. In many of its reactions it resembles albumin, for which it is apt to be mistaken, but is not coagulated by heat. It is precipitated by alcohol, alum, dilute mineral acids, and by certain organic acids, including acetic and citric. If urine containing mucin be poured on to the surface of acetic acid *saturated* with salt, or on to a strong solution of citric acid, a cloud appears at the junction of the two layers. On the other hand, mucin is soluble in saline solutions of moderate strength, and Purdy actually adds brine to urine in quantity sufficient to raise its density to 1035, in order to prevent the precipitation of mucin when acetic acid is subsequently added. In the nitric acid test for albumin the haze due to mucin appears above and distinct from that produced by albumin.

Salkowski and Leube test for mucin by treating the urine with two measures of nearly absolute alcohol, separating the precipitate by filtration, and redissolving it in water. The resultant solution gives with acetic acid a cloud which is insoluble in excess, but soluble in hydrochloric or nitric acid. Mucin gives the violet biuret reaction with caustic alkali and cupric sulphate, and is completely precipitated by lead acetate.

Mucin may be separated from pus by precipitating the latter by mercuric chloride. On adding acetic acid to the filtrate the mucin is precipitated. Pus is characterised by forming a gelatinous mass with alkalies, which reagents give no reaction with mucin.

There are several varieties of mucin. They all appear to have the constitution of glucosides, being compounds of a proteid (probably variable, but generally a globulin) with animal gum, which, by boiling with dilute sulphuric acid, yields a reducing unfermentable sugar.

In certain forms of liver-disease it has been found that the urine gives precipitates which may be mistaken for those of albumin, but which are in reality *bile-pigments*. It has been found, however, that these can be got rid of by previous treatment with acetic or a dilute mineral acid. Grocco, therefore, recommends that all samples of urine likely to contain such matters should be first treated with 2 or 3 per cent. of concentrated acetic acid, set aside in a cool place for two or three hours, and then filtered, before applying the ordinary tests. The precipitate thus formed by the addition of acetic or dilute mineral acids is soluble in alcohol, and does not give the biuret reaction.

THE NITROGENISED CONSTITUENTS OF URINE.

THE determination of the nitrogen contained in urine is often of great physiological and pathological interest, since the whole of the nitrogen contained in the effete nitrogenised tissues and in the food digested is ultimately eliminated by the kidneys.

About 90 per cent. of the total nitrogen contained in normal human urine exists in the form of urea, the remainder being divided between uric acid, hippuric acid, xanthine, creatinine, &c.[1] In the urine of herbivorous mammals the uric acid is replaced by hippuric acid, while the nitrogen of birds and reptiles is eliminated chiefly in the form of uric acid instead of as urea.

As urea is the predominant nitrogenous constituent of normal human urine, it is evident that for many purposes its determination will afford sufficient infor-

[1] The result of a large number of observations by Russell and West (*Proc. Royal Soc.*, xxx. 439) on various cases of disease was to prove that the relation of the ureal to the total nitrogen of urine is approximately constant, except in rare cases of acute yellow atrophy of the liver; and even in these it is doubtful whether the observed replacement of the urea by leucine and tyrosine is a constant phenomenon. In a case of acute fatty atrophy of the liver the urea was still normally formed, while leucine and tyrosine were absent. The following table shows the percentage of the total nitrogen existent as urea, according to the observations of Russell and West:—

Pneumonia (6 cases), 90 per cent.; jaundice (Case 1), 85·7; jaundice (Case 2), 90·2; albuminuria (2 cases), 86·0; collected cases, 93·8; dieted cases, 90·1; and mean of all, 89·3 per cent. The mean, excluding the jaundice and albuminuria cases, was 91·3 per cent.

mation as to the amount of nitrogen passing away in the urine.

Albumin and its allies are not present in normal urine, at least not in estimable amount, and their detection and determination in the pathological excretion have already been fully considered (page 105 *et seq.*).

Determination of the Total Nitrogen in Urine.

In certain cases it is desirable to ascertain the total amount of nitrogen existing in urine, without differentiating between the different modes of combination. The total amount of nitrogen is best ascertained by the well-known Kjeldahl method, which is based on the fact that nearly all forms of nitrogenised organic matter yield their nitrogen in the form of ammonia when heated strongly with concentrated sulphuric acid. Urea readily undergoes this conversion, and the decomposition of uric acid presents no difficulty. Albumin is split up primarily into glycocine, leucine, tyrosine, and probably other amido-compounds; but these, on further heating with strong sulphuric acid, yield all their nitrogen in the form of ammonia. Gunning finds the destruction of the organic matter to be much facilitated by adding some solid potassium sulphate, which raises the boiling point of the sulphuric acid.[1]

The conversion of the nitrogen into ammonia having been effected by the foregoing treatment, excess of caustic soda is added and the ammonia distilled off; an addition of sulphide of alkali-metal

[1] Potassium permanganate has been employed, but it is now recognised that its use is unnecessary and liable to lead to loss from oxidation of the ammonia formed. W. F. K. Stock effects rapid and complete oxidation of the organic matter by adding manganese dioxide to the hot acid. This plan is not available when the ammonia formed is to be decomposed with hypobromite, as described in the text. The use of mercury or mercuric oxide, which is a convenient means of effecting oxidation, results in the formation of a mercur-ammonium compound not decomposed by the reagent.

being made if mercury has been employed, in order to ensure the decomposition of mercur-ammonium bases.

The ammonia in the distillate is finally determined by titration with standard acid, and the amount found calculated into its equivalent of nitrogen.

The estimation of nitrogen by this process requires the observance of numerous precautions to ensure accuracy.

In a modified process, which presents fewer difficulties than the foregoing, and gives results sufficiently accurate for the ordinary purposes of urinary analysis, the nitrogen is first converted into ammonia by strongly heating with sulphuric acid, and the ammonia then decomposed by sodium hypobromite, the nitrogen evolved being measured in the form of gas. When treated with an alkaline hypobromite, ammonia compounds are decomposed in accordance with the following equation :—

$$3NaBrO + 2NH_3 = 3NaBr + 3H_2O + N_2.$$

This reaction, which was first applied by W. Knop, under favourable conditions occurs very promptly and completely; but in order to adapt the method to the requirements of urinary analysis, the manipulative details require careful arrangement. They have been thoroughly worked out in the author's laboratory, and by operating in the following manner fairly good determinations of the total nitrogen of urine can be obtained in about one hour.[1]

Twenty-five c.c. of the urine to be examined should be treated in a porcelain basin with 10 c.c. of strong sulphuric acid, and the liquid kept gently boiling until the volume is reduced to about 10 c.c. and

[1] A process on the same lines has been described by Petit and Monfet (*Jour. Pharm. und Chem.*, 1893, page 297), but their method of manipulating is different in many respects from that employed by the author. Both modifications are liable to give results below the truth.

white fumes of sulphuric acid are evolved.[1] The liquid is then allowed to cool, and carefully transferred to a pear-shaped flask, the basin being rinsed with a few drops of water. The flask is placed in an inclined position, to prevent loss by spurting, and the contents kept in gentle ebullition. If excessive frothing occur, it may be moderated by adding a small fragment of paraffin-wax (candle). When the frothing has ceased, about 5 grammes of potassium sulphate should be added and the flask heated strongly until the liquid is colourless or only a very pale yellow. The contents of the flask are then allowed to become cold, when about 20 c.c. of water is added very cautiously and a few drops at a time, agitating the liquid by a rotatory movement between each fresh addition. A highly concentrated solution of caustic soda, made by dissolving the alkali in about an equal weight of water, is now added gradually with constant agitation, until the sulphuric acid is nearly neutralised. This point may be ascertained by means of litmus-paper, or a few drops of litmus or phenol-phthalein solution may be added to the contents of the flask.

The neutralised liquid, which will measure about 80 c.c., is now diluted to exactly 100 c.c. with water, and thoroughly mixed by agitation. Ten c.c. of the solu-

[1] As a rule, the quantity of sulphuric acid prescribed is amply sufficient for the decomposition of the solids of 25 c.c. of urine. In the case of highly saccharine urine, however, the sugar chars and forms a black pasty mass, which cannot be readily transferred to the flask. In such a case, a further addition of sulphuric acid (5 to 10 c.c.) should be made, and the heating continued till the greater part of the carbonaceous matter is oxidised. It is important in all cases to avoid the use of an excessive amount of sulphuric acid, or so large an amount of soda must be employed to neutralise it, and so large a volume of water added to retain the salts in solution, that the measure of the neutralised liquid cannot be kept within 100 c.c., or indeed within any reasonable limits. On the other hand, less than 10 c.c. of acid is an inconveniently small volume to heat and manipulate. Hence it is desirable to adhere to the quantities of urine and acid prescribed in the text, and take an aliquot part of the neutralised liquid for treatment with hypobromite.

tion, representing 2·5 c.c. of the original urine, is now treated with the alkaline hypobromite reagent used for the determination of urea (page 141). The manipulation is the same as is described in the section on "Urea," and one of the forms of apparatus therein referred to may be employed. By far the most convenient apparatus for the purpose is, however, that shown in fig. 7. Ten c.c. of the neutralised solu-

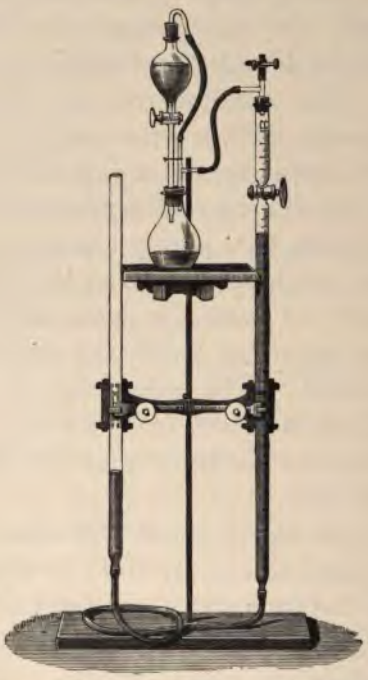


Fig. 7.—Nitrogen-Evolution Apparatus.

tion from the sulphuric acid treatment being placed in the flask, 25 c.c. of the hypobromite reagent should be poured into the separator, and the connections made as shown in the figure. The nitrometer should be filled to the tap with water or (preferably) brine, or, for very accurate experiments, mercury may be advantageously used. The apparatus being

adjusted, and the clip at the top of the nitrometer-cup having been momentarily opened to equalise the pressure in the cup with that in the flask, the tap of the nitrometer is opened, and the hypobromite solution then allowed to flow gradually into the flask. After adding about 10 c.c., the separator-tap should be closed and the flask agitated. A further addition of hypobromite is then made, the flask again agitated, and this treatment repeated until no further evolution of nitrogen takes place. As a rule, 10 c.c. of the reagent is sufficient to complete the reaction, which occurs very promptly and completely.[1] The flask is now allowed to acquire the temperature of the room, when the liquid in the nitrometer-tube is brought to the same level with that in the reservoir-tube, and the volume of nitrogen read off. If less than 20 c.c. of gas has been evolved, the process may be advantageously repeated on 20 c.c. or more of the neutralised liquid from the sulphuric acid treatment.

From the number of cubic centimetres of nitrogen evolved, v, the corresponding weight in milligrammes, W, may be calculated by the following formula, in which p represents the barometric pressure in millimetres; w the tension of aqueous vapour at

[1] The following figures, obtained in the author's laboratory by G. B. Brook, show that the process gives tolerably good, but somewhat low, results with solutions of pure ammonium sulphate, and that the reaction is fairly complete unless the dilution is excessive.

	Volumes of Solutions employed.					Percentage of Total Nitrogen evolved.
	Soda.	Bromine.	Water.	Am. Sulphate.	Total.	
A.	25 c.c.	2·5 c.c.	none c.c.	5 c.c.	32·5 c.c.	100·0
B.	25 ,,	2·5 ,,	20 ,,	5 ,,	47·5 ,,	98·1
C.	25 ,,	2·5 ,,	30 ,,	5 ,,	62·5 ,,	97·1
D.	25 ,,	2·5 ,,	50 ,,	5 ,,	82·5 ,,	92·7

If preferred, the hypobromite reagent may be placed in the flask and the neutralised solution added to it. In this case, a layer of water should be floated on the liquid in the separator, so as to rinse it completely into the flask.

the temperature at which the gas was measured ; and t the temperature in centigrade degrees :—

$$W = \frac{v \times (p - w)}{273 + t} \times 1{\cdot}251.$$

When strictly accurate results are not required, the corrections for temperature, pressure, and tension of aqueous vapour may be omitted and the calculation much simplified. Thus, the volume occupied by 28 milligrammes (= 0·028 gramme) of moist nitrogen at 16° C. (= 61° F.) and 762 millimetres (= 30 inches) pressure is 24 c.c. From this it follows that the grammes of nitrogen contained in 100 c.c. of urine can be calculated by the following equation, in which G represents the number of c.c. of gas evolved, and U the volume (in c.c.) of the original urine represented by the neutralised liquid used :—

$$N = \frac{G \times 28 \times 100}{U \times 24 \times 1000} = \frac{G \times 7}{U \times 60}.$$

Thus if the gas evolved from a measure of the neutralised liquid corresponding to 5 c.c. of the original urine measured 38·2 c.c., the sample contained 0·891 gramme of nitrogen per 100 c.c.

$$N = \frac{38{\cdot}2 \times 7}{5 \times 60} = \frac{267{\cdot}4}{300} = 0{\cdot}891.$$

This figure, multiplied by 4·375, will give the grains of nitrogen per fluid ounce of the urine ; or, if divided by the specific gravity of the sample (water = 1·000), the actual percentage by weight of nitrogen contained in the urine will be obtained.

It is evident that as Kjeldahl's process is applicable to the determination of the nitrogen of urea, uric acid, creatinine, albumin, &c., as they exist in urine, it is equally applicable to its determination in these substances when in an isolated state. Thus the nitrogen

contained in a precipitate of *uric acid* may be ascertained, and the weight of uric acid itself calculated therefrom; compounds of *creatinine* may be subjected to the treatment and the base thus determined[1]; or the *albumin* may be precipitated from 100 c.c. of urine, washed, and treated (together with the filter containing it) with strong sulphuric acid.[1]

A volume of 24 c.c. of moist nitrogen, measured at the ordinary pressure and temperature, corresponds to :—

0·028	gramme of	Nitrogen;
0·034	,,	Ammonia;
0·060	,,	Urea;
0·084	,,	Uric acid;
0·358	,,	Hippuric acid; or
0·164	,,	Albumin.

Urea. Carbamide. CH_4N_2O; or, $CO(NH_2)_2$.

Urea exists ready-formed in the urine of mammals, and in blood, milk, and other animal fluids. It was first prepared synthetically by Liebig and Wöhler in 1828, being the first of the natural organic bodies obtained by a synthetic process.[2]

Urea forms transparent, colourless, four-sided, anhydrous prisms (fig. 8). It is somewhat hygroscopic. Urea is odourless, and possesses a cooling saline taste, resembling that of nitre. When heated

[1] The evolution-method cannot be recommended in the case of creatinine. Albumin requires prolonged treatment with sulphuric acid to effect complete conversion of the proximate products of decomposition into ammonia.

[2] This classical discovery affords an interesting example of re-arrangement of the atoms in the molecule. Both ammonium cyanate and urea have an elementary composition corresponding to the empirical formula :— CH_4N_2O. On evaporating an aqueous solution of ammonium cyanate at the temperature of boiling water, the salt suffers molecular change into urea, according to the equation :— $CN.O(NH_4)=CO(NH_2)_2$. The converse reaction occurs when an aqueous solution of urea is heated with silver nitrate. A white precipitate of silver cyanate is formed, soluble in boiling water, while the solution is found to contain ammonium nitrate :— $CO(NH_2)_2+AgNO_3=CN.OAg+(NH_4)NO_3$.

to 132° C. it melts, and at a higher temperature decomposes with evolution of ammonia and ammonium cyanate, leaving a residue of cyanuric acid, $C_3H_3N_3O_3$, which bears a much stronger heat without change.

Urea is soluble in an equal weight of cold water, and in a much less quantity of hot. It is also readily soluble in alcohol, and dissolves in amylic alcohol, but it is nearly insoluble in ether, and quite so in chloroform and volatile oils.

At the ordinary temperature, an aqueous solution of pure urea shows no tendency to change, and is not decomposed by boiling; but when heated with water

Fig. 8.—CRYSTALS OF UREA—*a*, Quadrangular prisms; *b*, Indefinite crystals, as deposited from alcoholic solutions.

under pressure urea undergoes hydrolysis, with formation of ammonium carbonate, $CH_4N_2O + 2H_2O = (NH_4)_2CO_3$. In the urine, where the urea is associated with putrescible organic matter, it readily undergoes a similar change, which is the cause of the alkaline reaction of putrid urine. The ammoniacal fermentation of urine has been found to be due to the action of an organised ferment (*Torula ureæ*) in the urine. This change is set up by contact with the stomachs of men, dogs, or rabbits, and has often been occa-

sioned in the bladder by the introduction of a septic catheter.

Urea also yields ammonia when fused with caustic alkali or ignited with soda-lime, a carbonate being formed at the same time. When heated with a strong mineral acid, urea similarly forms an ammoniacal salt, carbon dioxide being evolved.

Pure concentrated nitric acid combines with urea without decomposing it, but if the acid contain nitrous acid the urea is resolved into water, nitrogen, and carbon dioxide, according to the following equation, the reaction, however, being far from complete:—

$$CH_4N_2O + N_2O_3 = 2H_2O + 2N_2 + CO_2.$$

Chlorine, bromine, hypochlorites, and hypobromites decompose solutions of urea with evolution of nitrogen. The best practical method of determining urea in urine is based on this reaction (page 138).

The basic character of urea is well-marked, although its solutions exhibit no alkaline reaction to litmus. It forms a series of well-defined salts, some of which crystallise readily. Many of them are decomposed by water.

Urea Nitrate, $CH_4N_2O.HNO_3$, separates in crystals when moderately strong nitric acid is added to a concentrated aqueous solution of urea, and the liquid cooled. The compound forms brilliant white scales or plates, or, if the deposition is slow, prismatic crystals. When nitric acid and urea are brought together on a microscope-slide, and the reaction observed under a low power, the formation of obtuse rhombic octahedra is first noticed, the angles being *constantly* 82°. These octahedra change to rhombic and hexagonal tables, either separate or superposed (see fig. 9, *a*), but also having angles of 82°. For the formation of nitrate of urea from normal urine, it is sufficient to concentrate the liquid to about one-fourth of its

volume, filter after cooling from the precipitated urates, &c., and add nitric acid to the cold filtrate. Nitrate of urea is unalterable in the air. It is readily soluble in water, forming a solution of acid reaction and taste. It is also soluble in alcohol, but only very slightly in presence of nitric acid. Oxalic acid precipitates urea oxalate from concentrated solutions of the nitrate.

Fig. 9.—*a*, UREA NITRATE; *b*, UREA OXALATE.

Urea Oxalate, $(CH_4N_2O)_2.C_2H_2O_4$, is readily formed on mixing concentrated solutions of urea and oxalic acid. From urine it may be prepared by adding oxalic acid to the concentrated and filtered liquid. Urea oxalate forms thin crystalline plates (see fig. 9, *b*), usually grouped together, but sometimes in well-formed separate crystals. Its microscopic appearance is not unlike that of the nitrate of urea, but the forms are less characteristic, and the angles are different. Oxalate of urea is soluble with difficulty in cold water, but dissolves readily at a boiling heat. It is less soluble in a solution of oxalic acid than in pure water. The salt dissolves in 62 parts of alcohol, but is quite insoluble in amylic alcohol. Hence, if a solution of urea in amylic alcohol (such as will result

from evaporating urine to dryness, heating the residue with amylic alcohol, and filtering) be treated with a cold saturated solution of oxalic acid in amylic alcohol, urea oxalate is precipitated in small crystals. By warming the liquid until the crystals are redissolved and allowing it to cool, the salt is obtained in a state fit for microscopic examination. The process may be modified by treating the solution of urea in amylic alcohol with one of oxalic acid in anhydrous ether. Precipitation takes place abundantly and quickly, but the crystals are usually small and imperfect. The oxalic acid may be added in powder, the liquid heated and thoroughly cooled, and the excess of oxalic acid removed from the precipitate by treatment with anhydrous ether. The method is capable of being employed quantitatively. The amylic alcohol used in the process must not develop a red or brown colour with oxalic acid, and should be free from water and ethylic alcohol.

Urea Phosphate, CH_4N_2O,H_3PO_4, forms large, very soluble, rhombic crystals on evaporating pig's urine, or mixed solutions of urea and phosphoric acid.

A compound of urea with sodium chloride, of the formula $CH_4N_2O,NaCl,H_2O$, separates in brilliant rhombic crystals when mixed solutions of urea and common salt are evaporated. It sometimes crystallises from concentrated human urine.

On mixing a solution of urea with one of neutral mercuric nitrate, a white flocculent precipitate is obtained. This has a composition dependent on the concentration of the liquid, containing, according to the conditions of its formation, 1, $1\frac{1}{2}$, or 2 molecules of mercuric oxide to 1 of urea. If, however, the addition of the mercuric nitrate be continued as long as precipitation occurs, and sodium carbonate be added from time to time to neutralise the nitric

acid set free, the precipitate has the composition $CH_4N_2O,2HgO$. The end of the reaction is indicated by the yellow colour developed from the formation of basic nitrate of mercury. Liebig's method of determining urea, now chiefly of historical interest, was based on this reaction.

Urea is not precipitated by a solution of mercuric chloride. The addition of mercuric nitrate to a soluble chloride results potentially in the formation of mercuric chloride. As sodium chloride is present in urine, mercuric nitrate produces no precipitate of Liebig's compound in that liquid until sufficient has been added to react fully with the chloride present. On this fact Liebig based a method for determining chlorides in urine.

Mercuric acetate gives no precipitate with urea in the cold, and the separation is very incomplete on boiling.

For the recognition of urea in a weak aqueous solution Bloxam has suggested the following method:— If a nitrate be present, add a few drops of ammonium chloride solution, but if absent, acidulate the liquid with hydrochloric acid. Evaporate the solution to dryness in a watch-glass, and heat the residue cautiously as long as thick white fumes are evolved. Dissolve the cooled residue in a drop or two of ammonia, add a drop of barium chloride, and stir. If urea was present, crystalline streaks of barium cyanurate will be formed in the track of the glass rod.

Determination of Urea.

Various methods have been devised for the determination of urea in urine, but those dependent on the measurement of the nitrogen gas evolved by its decomposition are by far the most convenient, and sufficiently accurate for ordinary purposes. They are

based on the reaction between urea and a strongly alkaline solution of hypobromite of sodium, whereby sodium bromide, water, carbon dioxide, and free nitrogen are produced, according to the following equation :—

$$3NaBrO+CH_4N_2O = 3NaBr+2H_2O+CO_2+N_2.$$

The carbon dioxide (carbonic acid) gas is absorbed by the excess of caustic alkali employed, so that, under the conditions of the experiment, pure nitrogen gas is evolved.

According to the great majority of observers, the reaction of cold hypobromite solution of the strength commonly employed results in the evolution of from 92 to 93 per cent. of the nitrogen existing in the urea present.[1] The suppressed nitrogen has been found to suffer conversion into cyanate. On heating the liquid, some further evolution of gas occurs, but the theoretical production is never realised, and under some circumstances there is a tendency to error from evolution of oxygen. In presence of cane-sugar, a more complete evolution of the nitrogen occurs, and hence C. Méhu (*Bull. Soc. Chim.*, [2], xxxiii. 410, and *Jour. Chem. Soc.*, xxxviii. 681) has proposed always to add 10 parts of cane-sugar for each 1 of urea supposed to be present. Glucose induces a still more perfect evolution of the nitrogen, but is said to be apt to occasion the liberation of traces of gas even in the absence of urea. In consequence of the peculiar action of glucose, the evolution of nitrogen on treating diabetic urine with hypobromite reaches 99 per cent. of the total nitrogen present in the urea. Even in normal urine, the evolved nitrogen bears a greater

[1] According to T. G. Wormley (*Chem. News*, xlv. 27), under favourable conditions the whole of the nitrogen of urea is evolved as gas. J. R. Duggan (*Amer. Chem. Jour.*, iv. 47, and *Jour. Chem. Soc.*, xlii. 778) states that fully 99 per cent. of the nitrogen is evolved as gas if 5 c.c. of the urine be first mixed with 20 c.c. of a solution of 20 grammes of caustic soda in 100 c.c. of water, and 1 c.c. of bromine is subsequently added.

proportion to the total amount present in the urea than is the case when pure solutions of urea are operated on. This fact is commonly attributed to the liberation of nitrogen from the uric acid, creatinine, and other urinary constituents, which, though present in but small amount relatively to the urea, are able to exert a sensible influence on the proportion of gas evolved. For ordinary purposes, the error due to this cause is wholly unimportant, and it has even been contended that, as the usual object of determining urea is to obtain a measure of the nitrogenous waste, all nitrogenised constituents of the urine should, as far as possible, be determined. Of course this is directly effected by determining the total nitrogen in the manner described on page 128.

Méhu points out that the uric acid present in urine is rarely more than 2 per cent. of the urea, and the creatinine still less, and as these bodies only evolve a portion of their nitrogen when treated with hypobromite they are incompetent to produce the whole of the effect ascribed to them. He attributes the better yield of nitrogen obtained from normal urine to the extractive matter present, which acts more or less like sugar. Hence Méhu recommends the addition of sugar in every case, so as to ensure the evolution of practically the whole of the nitrogen in the gaseous state.

G. Esbach (*Bull. Soc. Chim.*, [2], xxiv. 632) states that pure glucose solution, whether boiling or not, evolves a small quantity of gas when treated with the hypobromite reagent, but that cane-sugar yields no gas. He finds that in aqueous solutions of urea, the excess over the 92 per cent. normally evolved varies with the quantity and nature of the sugar added, the strength of the urea solution, and composition, especially the free alkali of the hypobromite

reagent, the volume of gas being greater the more alkali there is present. For the proportions of glucose present in diabetic urine, the excess of nitrogen over 92 per cent. is sensibly proportional to the mass of the sugar, but this does not hold good for large quantities. Urea *added* to a true diabetic urine is stated to evolve only 92 per cent. of its nitrogen. Esbach concludes that sugar should not be added to urine before applying the hypobromite process.

A very large number of determinations of urea by the hypobromite method have been made in the author's laboratory, the process being varied in many cases with the view of ascertaining the best conditions under which to work. The results have shown that the method is by no means so constant as generally supposed, and that it is liable to give low results from undiscovered causes. In presence of glucose or cane-sugar great evolution of heat occurs, and it is probably this phenomenon which is the cause of the higher results obtained in presence of sugar. Dilution of the hypobromite reagent with water, or the use of less bromine, did not greatly affect the results, but a large excess of bromine was prejudicial.

The hypobromite solution employed for the decomposition of urea is prepared by dissolving 100 grammes of good caustic soda in 250 c.c. of water, and thoroughly cooling the liquid; 25 c.c. measure of bromine is then added, and the resultant solution preserved in a cool place.[1] The reagent does not keep very well, in consequence of the gradual occurrence of the reaction:— $3NaBrO = 2NaBr + NaBrO_3$. Hence it is preferable to prepare the solution, when required, by mixing 25 c.c. of the caustic soda solution with 2·5 c.c. of bromine. Considerable variations in the strength of the reagent do not materially affect the results.

[1] Or 3½ oz. of caustic soda, 9 oz. of water, and 1¼ fluid ounce of bromine.

Various forms of apparatus have been devised for effecting the reaction and collecting the evolved nitrogen, and a suitable arrangement is easily extemporised. Of the special forms, that devised by A. W. Gerrard (*Pharm. Jour.*, [3], xv. 464) is among the best, when no extreme degree of accuracy is aimed at. Gerrard's apparatus (fig. 10) consists of a graduated tube, which is connected with a second tube, serving as a reservoir, by means of india-rubber tubing. The top of the graduated tube is closed by a

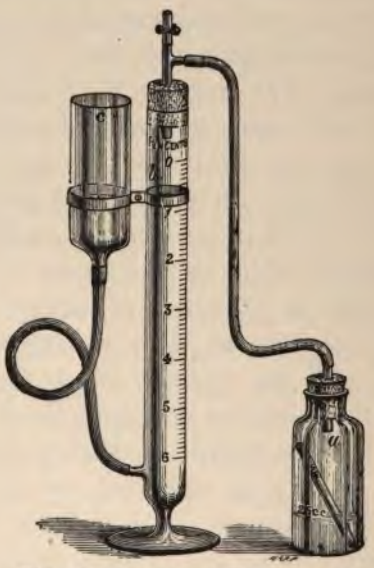


Fig. 10.—GERRARD'S UREOMETER.

caoutchouc stopper, through which passes a T-tube, one orifice of which is fitted with a short piece of india-rubber tubing closed by a clip, while the other communicates by a second piece of tubing with a bottle fitted with a perforated cork. In making the test, 25 c.c. of the hypobromite reagent should be poured into this bottle, and then a small test-tube containing 5 c.c. of the sample of urine cautiously placed in

it in such a manner as to avoid any contact between the urine and the reagent. The bottle is now connected with the graduated tube in the manner shown in the figure, the clip opened, and water poured into the reservoir-tube until, on suitably adjusting its height, the water stands at the zero-point in the measuring tube and at the same level in the reservoir, taking care that when this is effected but little water remains in the latter tube.[1]

The clip is then closed, and the bottle is tilted in such a manner as to allow the urine to mix gradually with the hypobromite solution, the bottle being gently agitated to promote the evolution of gas, which commences immediately and is complete in a few minutes. After five minutes, or preferably ten, the water in the measuring and reservoir tubes are brought to the same level by lowering the latter, when the volume of gas is read off.[2] In Gerrard's apparatus the tube is so graduated as at once to show the percentage of urea contained in the urine. If the urine contain more than 3 per cent. of urea, it is necessary to take 2·5 instead of 5 c.c., dilute it with an equal measure of water, and double the result obtained. With normal urine, the volume of nitrogen evolved under the foregoing mode of treatment is only 92 per cent. of that which would be yielded if the reaction formulated on page 139 were the only one which took place ; but as a fact, some 7½ to 8 per cent. is suppressed. Gerrard's apparatus is so graduated as to allow for this loss, and hence gives correct results when non-saccharine urine is under examination. But, in the case of urine containing much sugar, fully 99 per cent. of the ureal nitrogen

[1] This can be effected by raising the reservoir, and is necessary to make room for the water displaced by the gas subsequently evolved.

[2] In case the temperature of the room is greatly different from 15·5° C. (= 60° F.) the measuring tube should be immersed in water at that temperature, but for ordinary purposes, and under ordinary conditions, this precaution is unnecessary.

is given off, so that, to obtain the true amount of urea, the result indicated by Gerrard's apparatus should be multiplied by $92 \div 99 = 0{\cdot}93$.

In the absence of any specially constructed apparatus for the estimation of urea, a suitable arrangement can be readily extemporised. Thus, if preferred, the nitrogen may be simply collected in a graduated tube filled with and standing over water, but it is more convenient to employ a graduated tube open at the upper end, which is drawn out so as to admit of being readily connected with the india-rubber tube attached to the evolution flask. An inverted Mohr's burette, of a capacity of 50 c.c., immersed in a cylinder of water, answers very well. As long ago as 1877, A. Dupré (*Jour. Chem. Soc.*, xxxi. 534) recommended a tube of this kind, but furnished with a side-tube to which a clip was attached, which arrangement afforded some facilities in manipulating. These devices have all been superseded by the *nitrometer*, the employment of which for the purpose was first proposed by the author (*Jour. Soc. Chem. Ind.*, iv. 179).[1]

Fig. 11 shows the improved form of Lunge's nitrometer, furnished with a three-way tap, which enables communication between the nitrometer-tube and either the evolution-flask or the external air to be made at will, and materially facilitates the manipulation. The method of working is almost the same as with Gerrard's form of apparatus. The hypobromite solution is placed in the flask, the tube charged with the sample of urine is carefully introduced, the stopper firmly adjusted, and the india-rubber leading-tube

[1] Dupré's apparatus appears to have been the first ureometer in which the principle of the nitrometer was adopted. The same principle was utilised in Gerrard's arrangement, the description of which was published a few months before the appearance of the author's paper on "New and little-known applications of the Nitrometer"; but the apparatus had been used and sketched by the author some years previously.

attached to the nose of the three-way tap. The tap is then turned, so as to allow the nitrometer-tube to communicate with the external air, and the open reservoir-tube raised until the liquid in the graduated tube just rises to the zero-point, whereon the tap is closed. The tap is then turned so as to connect the flask with the nitrometer-tube, while cutting off the external air. The urine is then allowed to come gradually into contact with the reagent in the manner already described. When the reaction is complete, the liquid in the reservoir-tube is brought to the level of that in the nitrometer, and the volume of gas read off.

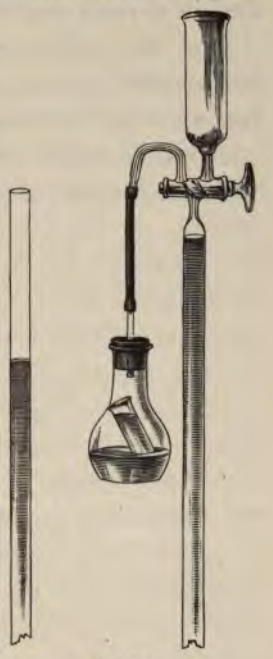


Fig. 11.

In the absence of a Lunge's nitrometer, the simpler form of apparatus devised by the author for assaying spirit of nitrous ether (*Pharm. Jour.*, [3], xv. 673), and known as "Allen's nitrometer," will answer every purpose. The arrangement is shown in fig. 12, but there is no absolute necessity for the T-tube and spring clip; though in their absence it is difficult to adjust the level of the liquid in the nitrometer-tube to the zero-point, and it becomes necessary to observe the position when the water in the open and graduated tubes stand at the same level.

In the modified form of apparatus shown on page 130, a separating funnel is substituted for the sample tube. This arrangement allows the urine and reagent to be brought together with any desired rapidity, or the bromine to be added to the soda and urine previously

mixed together in the flask.[1] These and other variations in manipulation are very valuable in researches, and in certain special applications of the nitrometer.

For all ordinary purposes, it is convenient and sufficiently accurate to fill the nitrometer with water, but mercury is sometimes used in research-work.

Another mode of using the nitrometer, and one which does not necessitate the use of an instrument

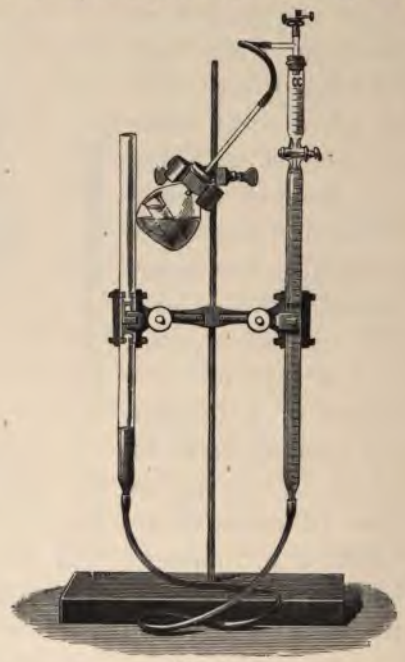


Fig. 12.

provided with a three-way tap or equivalent arrangement, has also been described by the author (*loc. cit.*). For this purpose the nitrometer is filled to the

[1] This mode of operating appears to be a valuable modification of the ordinary plan, since a sensibly more complete evolution of the ureal nitrogen is obtained, as was proved by experiments made in the author's laboratory by A. R. Tankard. Twenty-five c.c. of a 40 per cent. solution of caustic soda is placed in the flask, and 5 c.c. of the sample of urine added. The cork is then adjusted and a solution of bromine in aqueous bromide of potassium (20 per cent. solution) added gradually from the tapped separator. In each case the evolution of nitrogen occurred very promptly.

tap with strong brine, and the tap closed. Five c.c. of the sample of urine [1] should then be placed in the cup by means of a pipette, and allowed to flow into the tube by opening the tap cautiously. The last traces are rinsed in by a few drops of water. Ten c.c. of the hypobromite solution, previously diluted with an equal measure of water, is next added through the tap. The greater part of the nitrogen is liberated as soon as the reagent comes into contact with the urine. When the reaction has somewhat abated, a clip is placed on the india-rubber connecting-tube, and the nitrometer-tube vigorously shaken. When the reaction is completed, the clip may be removed, the liquids in the nitrometer- and reservoir-tubes brought to the same level, and the volume of gas observed. If the reading be rendered difficult from the formation of a persistent froth, this may be instantly destroyed by introducing a few drops of alcohol through the tap.

Instead of measuring the volume of gas evolved, it is sometimes convenient to measure the water displaced by it. This can be effected very simply by an arrangement devised by E. R. Squibb (*Ephemeris*, ii. 449). A still simpler device, and one which can be readily extemporised, has been devised by the author. It consists simply of the apparatus shown in fig. 3, page 47, with the addition of a test-tube to contain the sample of urine. One fluid ounce of the hypobromite mixture is placed in the feeding-bottle, and in the test-tube 2 fluid drachms (= 7·1 c.c.) of the sample of urine, together with a little simple syrup. The test-tube is placed inside the feeding-bottle, the stopper adjusted, and the contents of the tube allowed to mix with the hypobromite solution, just as when operating with the forms of apparatus

[1] If the volume of gas evolved exceed 40 c.c., a smaller measure of urine should be employed.

already described. The displaced water may be conveniently collected in a 2-oz. measure. Operating with $\frac{1}{4}$ oz. of urine, as prescribed above, each fluid ounce of water displaced represents 7 grains of urea per fluid ounce of the sample. Hence, if the measure collected were $1\frac{3}{4}$ oz. (= 14 drachms), the urine contained $12\frac{1}{4}$ grains per fluid ounce. The percentage of urea may be found by dividing $12\frac{1}{4}$ by 4·375, or multiplying it by 0·2286, which in the example taken would give 2·80 per cent.

In interpreting the results obtained by any of the modifications of the hypobromite method of estimating urea, it is unnecessary for ordinary purposes to take into account the barometric pressure, tension of aqueous vapour, or temperature at the time of making the experiment. By a fortunate coincidence, the increase in the volume of the gas, due to its being measured in a wet condition and at a temperature of 18° C. (= 65·4° F.), almost exactly compensates for the $7\frac{1}{2}$ or 8 per cent. of the total nitrogen ordinarily suppressed in the reaction. Thus Russell and West (*Jour. Chem. Soc.*, xxvii. 749) found that 0·100 gramme of urea gave off 37·1 c.c. of moist nitrogen at a temperature of 65° F. (= 18·3° C.). This, if dry, and at the standard pressure and temperature, would have measured 34·05 c.c., whereas the *total* nitrogen contained in the same amount of urea would measure 37·2 c.c. under the standard conditions, or nearly the volume actually obtained at the ordinary temperature. Hence 37·1 c.c. of gas, measured under the ordinary conditions of experiment, may be taken to represent 0·1 gramme of urea. Thus, if the cubic centimetres of gas evolved be multiplied by $\frac{1000}{371} = 2{\cdot}7$ (or, more exactly, 2·696) the product will be the number of milligrammes of urea in the volume of

urine employed; or, if 5 c.c. of urine were taken for the experiment, the volume of gas evolved, multiplied by 0·054 (or, more exactly, 0·0539) will be the grammes of urea (so-called "percentage") in 100 c.c. of the urine. The percentage thus found, multiplied by 4·375, will be the number of grains of urea contained in 1 fluid ounce of the urine.

If preferred, the above calculations can be combined, and the grains of urea per fluid ounce of the sample found at once by multiplying the measure of gas evolved from 5 c.c. of the sample by 0·236 (or, more exactly, 0·23585).

Instead of employing the foregoing factors, the content of urea per 100 measures and per fluid ounce can be found by reference to the following table, compiled by S. Henry Smith (*Pharm. Jour.*, [3], xxi. 294):—

Volume of Nitrogen in c.c. evolved from 5 c.c. of Urine.	Urea.		Volume of Nitrogen in c.c. evolved from 5 c.c. of Urine.	Urea.	
	Per cent.	Grains per oz.		Per cent.	Grains per oz.
1	·053	·231	29	1·56	6·82
2	·107	·468	30	1·61	7·04
3	·161	·704	31	1·67	7·30
4	·215	·94	32	1·72	7·52
5	·269	1·17	33	1·77	7·74
6	·323	1·41	34	1·83	8·00
7	·377	1·64	35	1·88	8·22
8	·431	1·88	36	1·94	8·48
9	·485	2·12	37	1·99	8·70
10	·539	2·35	38	2·04	8·92
11	·592	2·59	39	2·10	9·18
12	·646	2·82	40	2·15	9·40
13	·700	3·06	41	2·21	9·66
14	·754	3·29	42	2·26	9·88
15	·808	3·53	43	2·31	10·10
16	·886	3·87	44	2·37	10·36
17	·916	4·00	45	2·42	10·58
18	·975	4·26	46	2·47	10·80
19	1·02	4·46	47	2·53	11·06
20	1·07	4·68	48	2·58	11·28
21	1·13	4·84	49	2·64	11·55
22	1·18	5·16	50	2·69	11·76
23	1·24	5·42	51	2·74	11·98
24	1·29	5·64	52	2·8	12·25
25	1·34	5·86	53	2·85	12·46
26	1·40	6·12	54	2·91	12·73
27	1.45	6·34	55	2·96	12·95
28	1·50	6·56	56	3·01	13·16

It must be borne in mind that the foregoing factors and table are intended for use with non-saccharine urine. As already stated, the evolution of the nitrogen of urea is more complete in the presence of sugar than in its absence, and hence the results yielded when diabetic urine is treated with hypobromite should be multiplied by the factor 0·93 to obtain more correct results. An alternative plan is to add some simple syrup or honey (from 3 to 5 c.c.) to the urine in the sample-tube. Under these circumstances, 40 c.c. of nitrogen (against 37·1 c.c. in the absence of sugar) are yielded by 0·1 gramme of urea. Hence if 5 c.c. of urine be the volume employed, each c.c. of gas evolved represents 0·05 per cent. of urea in the sample. Thus, if the nitrogen measure 26·4 c.c., the sample contains 26·4 $\times$ 0·05 = 1·32 per cent. of urea.

The author's experience of the hypobromite method of estimating urea, under a great variety of conditions, and with the utmost care to obtain accurate results, leads him to the conclusion that the process is by no means so accurate, nor even so constant in its indications, as is commonly supposed. But it is undoubtedly a method of great practical utility, and one which is capable of doing excellent service in the hands of intelligent operators. Its readiness of application, and the great rapidity with which fair approximations can be obtained, specially adapt it to the requirements of the physician, who might employ it with advantage far more frequently than is the case at present.

INFLUENCE OF VARYING CONDITIONS ON THE EXCRETION OF UREA.

The amount of urea excreted varies considerably with the diet, being increased by nitrogenous foods. The weight of urea excreted per diem by an adult man on mixed diet ranges from 25 to 40 grammes,

the average being about 33 grammes (= 500 grains). On a diet poor in proteids the excretion of urea may fall to 15 to 20 grammes, while on a flesh diet the daily output may rise to 100 grammes. The proportion of urea in human urine averages about 2 per cent., but dog's urine is stated to contain 10 per cent.

A large excretion of urea, if long continued, points to increased tissue-metabolism or to surplus nitrogenous ingesta, but a temporary increase may be simply due to increased urination. Similarly, diminished excretion of urea may be due to diminished metabolism or to retention of urea in the system (as in uræmia).

A great number of observations have been recorded of the influence of drugs, diseases, and other conditions on the proportion of urea excreted. The results have been classified by W. D. Halliburton (*Chemical Physiology and Pathology*, 1891) as follow:—

An increased excretion of urea occurs:—

1. After administration of dilute sulphuric acid, potassium chloride, ammonium salts (especially with food), small doses of phosphorus, arsenic, antimony, morphine, codeine, or large doses of quinine.

2. After poisoning by phosphorus or arsenic.

3. From application of cold to the skin; after hot baths; from increase of oxygen inhaled; from excessive muscular work.

4. In diseases, as at the commencement of acute febrile diseases, up to the acme of the fever; during the paroxysms of intermittent fever (ague); in diabetes.

A decreased excretion of urea occurs:—

1. After administration of small doses of quinine.

2. During the sinking of the fever in acute febrile diseases; in most chronic and debilitating diseases (anæmia, syphilis, phthisis, dropsical affections, &c.); towards the fatal termination of most diseases (5 to 6 grammes daily); in uræmia (when the excretion may

entirely cease); in diabetic coma; and in all degenerative changes of the liver, especially in acute yellow atrophy.

Creatinine. Methyl-glycocyamidine.

$$C_4H_7N_3O;\ \text{or,}\ NH:C\left\{\begin{matrix}N(CH_3).CH_2\\NH.CO\end{matrix}\right\}.$$

Creatinine is an anhydride of creatine, $C_4H_9N_3O_2$, and is produced from the latter body with great facility.[1] Creatinine occurs constantly in normal human urine, the amount varying, according to Voit, from 0·5 to 4·9 grammes per diem, according to the quantity of proteids eaten. The proportion is not diminished by fasting, but is said to be increased in typhus, intermittent fever, pneumonia and tetanus, and diminished in convalescence from acute diseases, anæmia, chlorosis, paralysis and phthisis. Creatinine has been found in sweat and the muscles of fishes, and G. S. Johnson has isolated creatinine, or a modification of it, from the flesh of a healthy cow.

[1] CREATINE; Methyl-glycocyamine; or Methyl-guanidine-acetic acid.

$$C_4H_9N_3O_2;\ \text{or,}\ NH:C\left\{\begin{matrix}N(CH_3).CH_2.COOH\\NH_2\end{matrix}\right\}.$$

Creatine is a constant constituent of muscle-substance, to the extent of 0·2 to 0·3 per cent. of its weight, and is most conveniently prepared from Liebig's extract of meat. Creatine has also been found in nerve-tissue, and probably occurs in minute traces in urine and other animal fluids. But its isolation from these does not prove its pre-existence therein, since it is very easily formed by the dehydration of creatinine, into which body, on the other hand, creatine is very readily changed. Thus, on evaporating a solution of creatine with the calculated amount of dilute sulphuric acid, it yields a residue of creatinine sulphate. A similar change occurs very readily on boiling creatine with dilute hydrochloric acid, and the resultant creatinine can be readily recognised by conversion into the zinc salt.

Fig. 13.—CREATINE (after Frey).

Creatine crystallises in transparent rhombic prisms (fig. 13) containing water. It is sparingly soluble in cold water to form a slightly bitter solution, neutral to litmus. It reduces alkaline picric acid solution gradually in the cold, but immediately on boiling.

Creatinine may be isolated from human urine by Liebig's process, which consists in exactly neutralising the liquid with milk of lime, and adding calcium chloride as long as calcium phosphate continues to be precipitated. The filtrate, which should be neutral or very faintly acid, is evaporated to a small bulk, and the crystals of common salt, &c., removed. Thirty-two parts of the mother-liquor are treated with one part of zinc chloride in very concentrated solution, and the whole left for several days. The creatinine-zinc chloride which separates in nodules is washed with a little cold water, and then with alcohol. It is then boiled with recently precipitated lead hydroxide, the filtrate evaporated, and the residue digested with absolute alcohol, which dissolves the creatinine leaving any creatine insoluble.

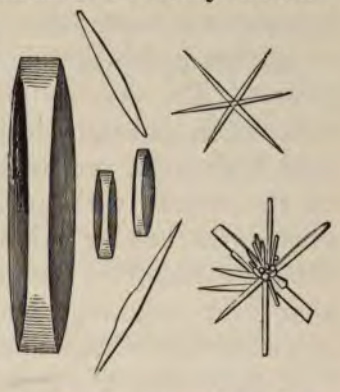


Fig. 14.—CREATININE (after Frey).

G. Stillingfleet Johnson prepares creatinine by a modification of Maly's process.[1] He treats a large volume of urine with 5 per cent. of its volume of a saturated aqueous solution of sodium acetate and 25 per cent. of saturated mercuric chloride solution. The precipitate which forms is filtered off immediately, and the filtrate left at rest for forty-eight hours. The creatinine separates in microscopic spherical masses of the mercuric chloride compound, which is filtered off, washed with cold water, and decomposed by sulphur-

[1] Maly (*Ann. Chem. Pharm.*, clix. 279) recommends a preliminary concentration of the urine by heat, and precipitation by basic acetate of lead before adding mercuric chloride. Johnson's modification is a marked improvement on this, as it gives a purer product and wholly avoids the use of heat, which is an essential condition if it be desired to obtain unchanged creatinine.

etted hydrogen. The filtered liquid is decolorised with purified animal charcoal, and concentrated by spontaneous evaporation over sulphuric acid, when creatinine hydrochloride separates in brownish-yellow prisms. The crystals are redissolved in 15 parts of cold water, and the solution treated with an excess of lead hydroxide, prepared by precipitating a solution of the acetate with ammonia. The mixture is well stirred for twenty minutes and then filtered. The filtrate is free from lead and chlorine, and on evaporation over sulphuric acid in a vacuum yields free creatinine in the form of efflorescent crystals containing $C_4H_7N_3O + 2H_2O$. If this creatinine be dissolved in hot water the crystals obtained on spontaneous evaporation are anhydrous tables, which, according to G. S. Johnson, are not chemically identical with the efflorescent form, but reconvertible into it by modifying the process of solution. The following table shows the results obtained by Johnson by the recrystallisation of "artificial" urinary creatinine (*Proc. Royal Soc.*, xliii. 526), reproduced from creatine which had been prepared by boiling urinary creatinine with water :—

Nature of Crystals.	Solvent.	Evaporation.	Product.
Effloresced Kα . .	Water at 60° C.	In vacuo.	Tabular Kβ.
Tabular creatinine α	Water at 60° C.	In vacuo.	Efflorescent Kβ.
Effloresced Kα . .	Water at 100° C.	In vacuo.	Tabular Kα (anhydrous).
Tabular Kβ (anhydrous) . . .	Cold water.	In vacuo.	Efflorescent Kα.
Efflorescent Kβ .	Cold water.	In vacuo.	Efflorescent K .

Creatinine as ordinarily obtained crystallises in oblique rhombic prisms and stellate forms (fig. 14). It dissolves in about 12 parts of cold water, and is also soluble in alcohol, but almost insoluble in ether.

The aqueous solution of creatinine is, according to some observers, neutral, but according to others

alkaline.[1] The solution readily undergoes change with formation of creatine, especially if ammonia, oxide of lead, or other base be present. By prolonged boiling with caustic alkali, creatinine is completely decomposed.

By boiling with baryta-water, creatinine is hydrolysed to ammonia and methyl-hydantoin,

$$C(NH)\begin{cases} N(CH)_3.CH_2 \\ NH\text{——}CO \end{cases} + H_2O = NH_3 + CO\begin{cases} N(CH_3).CH_2 \\ NH\text{——}CO \end{cases}.$$

Boiled with water and mercuric oxide, it gives methyl-guanidine.

Creatinine yields a series of crystallisable salts. The *hydrochloride*, BHCl, crystallises in prisms from alcohol or in laminæ from water. It unites with zinc chloride to form the double salt $ZnCl_2,2BHCl$. This is very soluble in water and alcohol, and must not be mistaken for the compound $ZnCl_2,2C_4H_7N_3O$, which is one of the most characteristic salts of creatinine. *Creatinine-zinc chloride* is obtained by mixing concentrated aqueous or alcoholic solutions of zinc chloride and creatinine, or by adding sodium acetate to the solution of the double hydrochloride. It forms oblique rhombic prisms or small needles, which have a tendency to form rosettes or warty concretions. The crystals are soluble in about 54 parts of cold or 27 of boiling water. They are insoluble in absolute alcohol, and require 9217 parts of alcohol of 98 per cent., or 5734 of alcohol of 87 per cent.

Mercuric chloride gives a white, curdy precipitate in strong solutions of creatinine, but the separation is not perfect unless sodium acetate be added, or mercuric acetate substituted for mercuric chloride. On allowing such a mixture to stand at the ordinary temperature, the compound is gradually deposited in

[1] A strongly alkaline sample leaves an alkaline ash on ignition, proving the presence of mineral impurity. Salkowski finds creatinine quite free from alkaline reaction, but it liberates ammonia from ammoniacal salts on boiling.

microscopic spherules. This reaction is applied by G. S. Johnson to the isolation of creatinine from urine (*Proc. Royal Soc.*, 1888, xliii. 507). The compound is almost insoluble in cold water, and is decomposed with partial reduction of the mercury by hot water. It is readily soluble in dilute hydrochloric acid, but is nearly insoluble in acetic acid. Johnson attributes to the spherical mercury compound the formula $C_{16}H_{28}N_{12}Hg_7Cl_{10}$, and suggests the following constitution :—$4(C_4H_5Hg''N_3O,HCl),3HgCl_2 + 2H_2O$. Experiments on specimens of the spherical salt prepared in the author's laboratory, both from urine and from pure creatinine prepared by Johnson's process (page 154), do not fully confirm this formula. The proportion of chlorine, in particular, varies materially, a fact which points to the presence of HgO, and possibly of Hg_2Cl_2, in some preparations.

From a concentrated solution of creatinine, silver nitrate precipitates crystals of the compound, $C_4H_7N_3O,AgNO_3$. Mercuric nitrate does not precipitate a dilute solution of creatinine till excess of sodium carbonate is added, when $B_2Hg(NO_3)_2,HgO$ is thrown down as a crystalline precipitate.

Creatinine possesses marked reducing properties. The mercury of the spherical salt above described is reduced, even in the cold, partly to the mercurous state and partly to metal on adding caustic alkali. Contact with boiling water produces a similar change.

Creatinine reduces Fehling's solution on boiling, the blue liquid changing to yellow, but no cuprous oxide separates. Creatinine appears also to prevent the separation of a precipitate when glucose is present, and hence exerts an interfering action on the application of Fehling's solution to the detection of dextrose in urine (compare page 65). Pavy's solution is reduced by creatinine without precipitation, and the reagent

may be used for its determination. According to G. S. Johnson, the reducing power of creatinine obtained direct from urine is greater than that of the base prepared from creatine (compare page 154). Thus he finds 12 grammes of tabular creatinine-α from urine to have a cupric oxide reducing power equivalent to 12 grammes of glucose; that is two molecules of this creatinine equal one of glucose in reducing power; against $2\frac{1}{2}$ molecules required of creatinine prepared from creatine.

Phosphomolybdic and phosphotungstic acids produce micro-crystalline precipitates in solutions of creatinine acidulated with nitric or hydrochloric acid. By treating the precipitates with baryta, free creatinine is obtained.

If a concentrated solution of picric acid be added to normal human urine a small crystalline sediment is gradually formed. On separating this, and treating it with hot water, uric acid remains, while the greater part dissolves. The soluble portion is a double picrate of potassium and creatinine, which forms lemon-yellow needles or thin prisms, readily soluble in hot water, sparingly in cold alcohol, and almost insoluble in ether. With dog's urine the precipitate produced by picric acid contains little or no uric acid, and the kynurenic acid present is not precipitated.

When a solution of picric acid is added to a solution of creatinine not more dilute than 1 in 3000, on adding a drop of dilute caustic alkali a deep red colour is produced, which is intensified by boiling the liquid. By this reaction the presence of creatinine can be recognised in the urine of man, dog, and rabbit. Acetone gives a similar but less intense colour. Glucose gives a similar reaction on heating. It is evident that the behaviour of creatinine with picric acid gravely affects the value of that reagent as a test for small quantities of sugar in urine.

T. Weyl (*Berichte*, 1878, page 228) has pointed out that if a few drops of very dilute solution of sodium nitroprusside be added to a solution of creatinine, and dilute caustic soda then added drop by drop, a fine ruby-red colour will be produced, which in a few minutes changes to an intense straw-yellow. If the liquid be now acidulated with acetic acid and warmed, it turns greenish and prussian blue separates. Guareschi recommends that 10 per cent. solutions of nitroprusside and caustic soda should be used. Krugenberg states that the reaction is best obtained by first adding caustic soda, and then a few drops of a concentrated solution of the nitroprusside. Salkowski confirms this. The reaction is very delicate, and can be obtained with a solution containing 0·03 per cent. of pure creatinine, or with urine containing 0·066 per cent. In applying the test to urine the absence of acetone should be insured by distilling off a portion, since that body gives a ruby-red colour with Weyl's test, though no blue colour can be obtained on acidulating, acetic acid merely restoring the yellow colour to red. According to Guareschi, a red colour is also yielded by hydantoin, methyl-hydantoin, and other compounds containing the group $N.CH_2.CO.N$. Creatine gives no reaction with Weyl's test unless the liquid be first boiled with a dilute acid, so as to convert it into creatinine. In this manner, Weyl demonstrated the presence of creatine in milk (*Berichte*, xi. 2175).

The determination of creatinine in urine is usually based on its isolation as creatinine-zinc chloride, which process is preferred by Neubauer. P. Grocco (*Chem. Centr.*, 1887, page 17) has described the following modification of this method of isolating creatinine from urine. The urine of twenty-four hours, kept acid by acetic acid, and preserved in ice if possible, is treated with milk of lime till only faintly acid, calcium

chloride added and the liquid evaporated, the reaction being maintained neutral or faintly acid by cautious addition of acetic acid. The residue is extracted with alcohol containing a little sodium acetate, and the filtered liquid treated with alcoholic zinc chloride and a little acetic acid, avoiding excess. If necessary, the alcoholic extract is decolorised with animal charcoal before adding the zinc reagent, and it should be cooled with ice. The purity of the precipitated creatinine-zinc chloride should be proved by a microscopic examination, with a high power, to make certain of its freedom from sodium chloride. It should be completely soluble in hot water. (Compare page 155.)

E. Salkowski operates in a very similar manner, but uses a smaller quantity of urine. He directs that 240 c.c. should be rendered alkaline by the cautious addition of milk of lime, and precipitated by calcium chloride. The volume is made up to 300 c.c., and the liquid filtered after ten minutes. 250 c.c. of the filtrate, representing 200 of urine, which must be feebly alkaline, is evaporated to about 20 c.c., and an equal measure of absolute alcohol added. This is subsequently diluted to 100 c.c. with alcohol, allowed to stand twenty-four hours, and filtered. To 80 c.c. of the filtrate zinc chloride is added, and the creatinine-zinc chloride collected after twenty-four hours.

Instead of weighing the compound of creatinine with zinc chloride, the contained creatinine may be deduced from the amount of ammonia produced on decomposing it with boiling concentrated sulphuric acid. For this purpose the precipitate should be dissolved in the minimum quantity of sulphuric acid, previously diluted with an equal measure of water, and the solution treated as described on page 128.

A method of isolating creatinine from urine, which, in the opinion of the author, is preferable to the zinc

process, is that based on its precipitation as the spherical mercuric compound (described on page 156), which, according to G. S. Johnson, contains about 20 per cent. of creatinine. Instead of weighing the precipitate, it may be washed with cold water, and decomposed by treatment with strong sulphuric acid as in Kjeldahl's process[1] described on page 127, the creatinine being deduced from the amount of ammonia obtained. This plan, in the opinion of the author, is preferable to weighing the mercury salt.

Attempts to determine creatinine by treating the zinc or mercury compound with alkaline hypobromite solution, as employed for the estimation of urea, have failed to give the author satisfactory results, the proportion of the total nitrogen evolved on treatment with the hypobromite being apparently dependent on conditions not yet mastered. Hence the method, which at first appeared very promising, cannot at present be recommended.

Uric Acid. Lithic Acid. $C_5H_4N_4O_3$.

Uric acid is one of the most constant and characteristic products of the metabolism of the animal organism. In its formation the nucleïn of the cell-nuclei is specially concerned. It exists normally as urate of sodium in human urine, the proportion present being greatly increased in cases of gout and rheumatism, when the urine contains large quantities of acid urate of sodium.[2] Traces of uric acid exist normally in the

[1] Kjeldahl's original method must be employed, as the modified process described on page 128 is not applicable in presence of mercury compounds.

[2] The amount of uric acid commonly excreted by an adult is usually stated to be about 8 grains (0·5 gramme) in the twenty-four hours, but the results of the more modern methods of determination lead to the conclusion that the diurnal quantity eliminated under normal conditions is from 20 to 30 grains (1·3 to 2·0 grammes). The deposition of urates from urine on cooling does not prove their presence in excessive amount.

During recent years, Haig and other observers have shown that uric acid

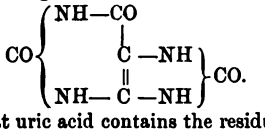
brain, lungs, liver, and spleen, and uric acid is also found in the saliva, gastric juice, sweat, &c. The merest trace exists normally in blood, but in cases of albuminuria, and especially of gout, the proportion becomes very appreciable. The so-called "chalk-stones," and other gouty concretions, commonly consist of the sparingly soluble acid sodium urate, while the buff-coloured sediment which frequently separates from human urine usually consists of the quadri-urate of sodium or ammonium. Acid urate of ammonium constitutes the greater part of the urinary excrement of birds ("guano"), while that of serpents and other terrestrial reptiles contains it in a still purer form. On the other hand, uric acid is nearly absent from the urine of herbivorous mammals, being replaced therein by hippuric acid.

The synthesis of uric acid has been effected[1] by Horbaczeweski by heating glycocine with ten times its weight of urea to about 230° C. :—

$$\underset{\text{Glycocine.}}{C_2H_5NO_2} + 3\underset{\text{Urea.}}{CH_4N_2O} = \underset{\text{Uric acid.}}{C_5H_4N_4O_3} + \underset{\text{Water.}}{2H_2O} + \underset{\text{Ammonia.}}{3H_3N}.$$

has, in all probability, a much wider bearing in pathology than had previously been supposed. Its connection with gout, rheumatism, and stone has long been recognised, though its relation to these complaints has been much misunderstood. Its relation to other affections (*e.g.*, according to Haig as a casual factor in migraine) is still more obscure.

[1] Another interesting synthesis of uric acid has been effected by Behrend and Roosen (*Berichte*, xxi. 999) by the reaction of etho-acetic ether and urea. From this synthesis the following constitutional formula, first proposed by Medicus, has been assigned to uric acid :—



This formula shows that uric acid contains the residues of two molecules of urea, and explains the fact that the decompositions of uric acid almost invariably yield either a molecule of urea or some derivative of urea, together with a second body which can by further treatment be converted into urea. Many of the decomposition-products of uric acid can indeed be prepared directly from urea. In view of the close relationship existing between urea and uric acid, it is not surprising that the foods which, in the mammal, cause an increased excretion of urea, in birds are converted into uric acid.

Conversely, when uric acid is heated under pressure to 170° with hydriodic acid, it yields glycocine, ammonia, and carbon dioxide.

Uric acid differs by an atom of oxygen from xanthine, a feeble base of wide occurrence in both the animal and vegetable kingdoms, and the physiological and pathological relations of which are but little understood.[1]

[1] XANTHINE, $C_5H_4N_4O_2$, is the typical member of a series of feebly basic bodies closely related to uric acid and to each other. Some of these compounds (*e.g.*, xanthine, hypoxanthine, guanine, carnine) occur in small quantity in normal urine and animal organs and tissues, and are normal products of the degradation of proteids. Other members of the group (*e.g.*, caffeine, theobromine, theophylline, and xanthine itself) occur in plants.

Hypoxanthine ($C_5H_4N_4O$), *xanthine* ($C_5H_4N_4O_2$), and *uric acid* ($C_5H_4N_4O_3$) all occur in normal urine. They differ from each other only by an atom of oxygen, but, notwithstanding this close relationship, they do not seem to be convertible, as has been alleged. *Heteroxanthine* ($C_6H_6N_4O_2$) and *paraxanthine* ($C_7H_8N_4O_2$) also occur in normal urine, and the former is said to be present in larger amount in the urine of anæmic persons.

The vegetable bases *theobromine* and *theophylline* are dimethyl-xanthines, $C_5H_2(CH_3)_2N_4O_2$, while *caffeine* has the constitution of a trimethyl-xanthine, $C_5H(CH_3)_3N_4O_2$.

Guanine, $C_5H_5N_5O$, a body abundant in Peruvian guano, has the constitution of an imido-xanthine; while *adenine*, $C_5H_5N_5$ (originally isolated from the pancreas), bears the same relationship to hypoxanthine.

Xanthine was originally discovered (Marcet, 1819) in a urinary calculus. It has been found in guano, and is a normal constituent of urine, especially during the use of sulphur-baths, and is present in minute quantities in various parts of the system. It has also been found in tea, lupines, malt-seedlings, yeast, &c., and has been produced synthetically.

In their chemical and physical characters, the xanthine bases present a close resemblance to uric acid. They have but a feeble affinity for acids, and their salts are mostly decomposed by water. Some of them (including xanthine itself) exercise an acid function in addition, and unite with bases. They are mostly very slightly soluble in cold water, and, except caffeine and theobromine, insoluble in alcohol, ether, or chloroform. They all yield white precipitates with phosphomolybdic acid, mercuric chloride, and ammoniacal lead acetate; and guanine and adenine are very perfectly precipitated by picric acid.

A general reaction of the xanthine bases is their precipitation from ammoniacal solutions by ammonio-nitrate of silver, as a gelatinous compound of the base with argentic oxide. On treating the precipitate with dilute nitric acid, crystalline compounds of the bases with silver nitrate are obtained, the xanthine compound containing $C_5H_4N_4O_2,AgNO_3$. The different solubility of these compounds in water and nitric acid affords a means of distinguishing and separating the xanthine bases from each other.

The xanthine derivatives (except caffeine and theobromine) are precipitated

As commonly obtained, uric acid is said to be anhydrous,[1] and forms small crystalline scales which are very apt to appropriate colouring matter. The microscopic

by cupric acetate, especially on heating. The precipitates are the cuprous salts of the bodies, that yielded by xanthine containing $Cu_2O,C_5H_4N_4O_2$. They may be produced by treating the neutral solution of the body with a mixture of cupric sulphate and sodium sulphite or thiosulphate, or by mixing the ammoniacal solution with Fehling's solution, heating to boiling, and gradually adding a solution of dextrose. Instead of cuprous oxide separating in the free state, it combines with the xanthine-derivative to form a white insoluble compound. Hence it is evident that the presence of xanthine and its allies, including uric acid, will prevent the detection of sugar in urine by Fehling's test to an extent dependent on the amount of the interfering body present. The fact is of considerable practical importance when small quantities of sugar are to be sought for, and therefore, in such case, Fehling's test should be applied in the modified manner described on page 62. If, instead of using dextrose as a reducing agent, the mixture of a xanthine base with Fehling's solution be treated with hydroxylamine hydrochloride, reduction of the copper to the cuprous state will occur in the strongly alkaline solution and at the ordinary temperature. P. Balke (*Jour. Pract. Chem.*, [2], xlvii. 537) employs the foregoing reaction for the determination of the xanthine derivatives, and for their isolation from flesh, malt, &c.

The proportion of xanthine derivatives (other than uric acid) ordinarily present in urine is extremely small, but there is reason to believe that, under circumstances not fully understood, their amount is much increased and may then be of pathological importance. For the extraction of xanthine from urine, a large quantity (5 to 10 gallons) should be treated by instalments of about 1 quart at a time with neutral lead acetate in powder, as long as a precipitate is produced. The liquid is filtered and sodium sulphate added as long as lead sulphate is thrown down, the liquid poured off or filtered from the precipitate, copper sulphate added, and the liquid boiled. The precipitate, which contains the xanthine derivatives as cuprous salts, is filtered off, washed, dissolved in dilute nitric acid, and excess of ammonia added, followed by silver nitrate. The precipitate, consisting of the argentic oxide compounds of xanthine, &c., is separated, suspended in hot ammoniacal water, and decomposed by sulphuretted hydrogen, the resultant silver sulphide filtered off, and the filtrate concentrated till the xanthine crystallises out. If, instead of decomposing the silver precipitate with sulphuretted hydrogen, it be boiled with nitric acid of 1·10 specific gravity, the silver nitrate compounds of hypoxanthine and adenine will crystallise out immediately on cooling, while those of xanthine, paraxanthine, and heteroxanthine will remain in solution, and may be recovered as the silver-oxide compounds by rendering the filtrate ammoniacal.

[1] When slowly deposited from dilute solutions, uric acid sometimes separates in large crystals containing $C_5H_4N_4O_3+2H_2O$. It is very probable that some of the ordinary forms of uric acid are hydrated. Thus Dr. James Edmunds finds that all crystals of uric acid, obtained by addition of hydrochloric acid to cold filtered urine, effloresce and break up on heating to 200° F. or on exposure in a desiccator at 60° F. over sulphuric acid for twenty-four hours. Preparations made by Dr. Edmunds show this in a marked manner.

appearance of uric acid is very variable, dumb-bell, whetstone, and lozenge-like forms being among the most characteristic (fig. 16, page 167). When precipitated from urine by adding hydrochloric acid, it commonly forms small, transparent, rhombic tablets, with a few elliptical and oblong plates; but the forms assumed greatly depend on the nature and amount of the pigments and other co-existing substances.

When quite pure, uric acid forms a white crystalline powder without taste or smell, and of a specific gravity ranging from 1·855 to 1·893. On heating, it decomposes without melting, giving off hydrocyanic acid and carbon dioxide, yielding a sublimate containing cyanuric acid, ammonium cyanate and urea, and leaving a carbonaceous residue.

Uric acid is nearly insoluble in water, requiring 15,000 parts of cold or 1800 of boiling water for its solution. Blarez and Deniges (*Compt. rend.*, civ., 1847) find that 100 grammes of water at 0° C. dissolve 2·0 milligrammes of uric acid; at 10°, 3·7; at 20°, 6·0; and at 100°, 62·5 milligrammes of uric acid. Uric acid dissolves in glycerin, but in alcohol and ether it is quite insoluble. Uric acid is soluble in solutions of the borates, phosphates, carbonates, acetates, and lactates of potassium and sodium, but not in solutions of the corresponding ammonium salts.

In strong sulphuric acid uric acid dissolves to form a crystallisable sulphate, which is decomposed by water, the uric acid being precipitated unchanged. When strongly heated with concentrated sulphuric acid, uric acid is broken up, the nitrogen being ultimately wholly converted into ammonia (see page 127).

Hydrochloric acid has neither solvent nor chemical action on uric acid. By the action of oxidising agents on uric acid a number of compounds of great theoretical interest are obtainable. These form two dis-

tinct series. The first, represented by alloxan, $C_4H_2N_2O_4$, are produced by acid oxidising agents, such as nitric acid. The second, of which allantoin, $C_4H_6N_4O_3$, is the type, result from the oxidation of uric acid in alkaline or neutral solution.

Hot dilute nitric acid converts uric acid into alloxantin, $C_8H_4N_4O_7$, a body which is also produced by the action of reducing agents on alloxan. Chlorine and bromine convert uric acid at ordinary temperatures into urea and alloxan. On heating, parabanic and oxalic acids are also produced. Hypobromites and hypochlorites cause the evolution of a portion of the nitrogen of uric acid in a gaseous state, but the reaction does not appear to be sufficiently constant to serve as a method of determining uric acid.

Uric acid suspended in pure water remains unchanged for a long time, but the addition of a very small quantity of decomposed urine causes its rapid and complete decomposition in hot weather, with formation of ammonium carbonate and other bodies.

DETECTION AND DETERMINATION OF URIC ACID.

Uric acid is commonly separated in the free state by adding excess of hydrochloric acid to its solution. When separated from urine in this manner, it forms a coloured deposit which adheres to the sides of the glass.[1] The best mode of operating is described in the sequel.

When isolated, uric acid is readily identified by its microscopic appearance, though the forms it assumes are very numerous. When deposited from urine or other impure solutions, dumb-bell, whetstone, and lozenge-like forms are among the most common and

[1] A drop of fresh urine, mixed with hydrochloric acid, may be observed under the microscope to deposit uric acid crystals in the course of a few minutes.

characteristic (fig. 16, *b* and *c*). A. E. Garrod has shown that the pigments of urine are especially concerned in modifying the forms assumed by the uric acid, and that the presence of excess of one particular pigment will produce a corresponding definite variation in the form of the crystals. Dr J. Edmunds has independently found that the forms assumed by uric acid greatly depend on the nature and amount of the co-existing substances. When precipitated from a solution of a pure urate by addition of hydrochloric acid, uric acid generally forms minute transparent rhombic plates (fig. 16, *a*). Large crystals are obtainable much more readily from urine or other impure solutions than from pure urates.

Fig. 15.—Crystals of Uric Acid.

A highly characteristic and delicate reaction is that known as the "murexide test," which is based on the behaviour of uric acid on oxidation. If uric acid, a urate, or even urine be treated with a few drops of strong nitric acid, and the liquid evaporated to dryness in porcelain at 100°, a yellowish or red residue will be obtained, which owes its colour to the formation of alloxantin, $C_8H_6N_4O_8$. On inverting the capsule over another containing ammonia, or otherwise subjecting the

residue to ammoniacal vapours, it acquires a magnificent purple colour, owing to the formation of murexide or ammonium purpurate, $NH_4.C_8H_4N_5O_6$. On now adding caustic soda, the purple becomes changed to blue, the colour disappearing on warming. Somewhat analogous reactions are given by caffeine, theobromine, guanine, and xanthine, but the differences do not allow of their confusion with uric acid.

Fig. 16.—Crystals of Uric Acid.—*a*, From decomposition of urates; *b*, from human urine; *c*, dumb-bell forms.

The nitric acid prescribed in the above test may be advantageously replaced by bromine-water, or the material to be tested may be evaporated with a few drops of strong hydrochloric acid and a minute crystal of potassium chlorate.

If uric acid be dissolved in a solution of sodium carbonate and a drop of the liquid placed on filter-paper previously moistened with silver nitrate, a yellow, brown or black spot will be produced, owing to the fact that silver carbonate is reduced by uric acid even at the ordinary temperature.

On adding a little Fehling's solution to a solution of uric acid in caustic soda, a greyish precipitate is formed, said to consist of cuprous urate; but with excess of the reagent, and on application of heat, red cuprous oxide separates.

Uric acid does not reduce a hot, alkaline solution of picric acid, which fact distinguishes it from creatinine, glucose, and other normal and occasional constituents of urine which do react with Fehling's solution.

For the *determination* of uric acid in urine a common practice is to add hydrochloric acid to the

previously concentrated sample, allow the liquid to stand in the cold for a few hours, and filter from the precipitated uric acid, which is subsequently washed with cold water and rectified spirit, dried, and weighed. But under the most favourable circumstances, and however carefully the process be conducted, the separation of the uric acid is incomplete, and the results consequently below the truth.

A preferable method of isolating the uric acid from urine is that based on the insolubility of the acid ammonium urate, $C_5H_3(NH_4)N_4O_3$, in a solution of ammonium chloride or sulphate. In the original process, which is due to A. P. Fokker (*Jour. Chem. Soc.*, xxviii. 1293; also Salkowski, *Fresenius' Zeitschrift*, xvi. 371), only a limited amount of ammonium sulphate was used, and hence a considerable correction was necessary for the solubility of the acid urate; but F. G. Hopkins (*Chem. News*, lxvi. 106) has pointed out that by saturating the liquid with ammonium chloride no such correction is required, and the time necessary for complete precipitation is much reduced. Hopkins prescribes the following procedure:—

To 100 c.c. (or 4 fluid ounces) of urine, finely powdered ammonium chloride is added in excess,[1] about 30 grammes (1 oz.) being necessary. When a small quantity of the salt remains undissolved, even after brisk stirring at intervals of a few minutes, saturation is sufficiently complete, even if complete solution occurs when the liquid recovers from the depression of temperature caused by the solution of the ammonium chloride. The solution is allowed to stand for two hours with occasional stirring, and is then passed

[1] When it is intended subsequently to decompose the precipitate by hydrochloric acid and weigh the liberated uric acid, it is essential that the ammonium chloride used should dissolve to an absolutely clear solution in water, since the quantity employed is very large relatively, and any insoluble matter would seriously vitiate the estimation of uric acid.

through a thin filter and washed twice with a saturated solution of ammonium chloride. When time is an object, the urine may be made alkaline with ammonia after saturation with ammonium chloride. The phosphates which are thus precipitated with the ammonium urate occasion no inconvenience, while precipitation is complete in ten minutes.

When a normal acid urine is saturated with pure ammonium chloride, the precipitate of acid ammonium urate, after being washed with the cold, saturated solution of ammonium chloride, yields a mere trace of ash on ignition, showing that no mineral salts are carried down. Of the ordinary constituents of urine, uric acid, xanthine, and certain pigments appear to be the only bodies precipitated. *Xanthine* is thrown down still more completely from ammoniacal solutions, but it is left in solution when the ammonium urate is subsequently decomposed by hydrochloric acid. Hypoxanthine and creatinine are not precipitated by ammonium chloride. Certain *pigments* are thrown down, so that the precipitate is always more or less coloured. *Hæmato-porphorin*, in particular, is very perfectly precipitated, but remains in solution when the urate is subsequently decomposed by acid.

The acid ammonium urate, isolated in the foregoing manner, admits of several alternative treatments, as follow :—

1. When it is desired to determine the uric acid by weight, the precipitate is rinsed off the filter with a jet of hot water, and the liquid heated just to boiling with excess of dilute hydrochloric acid. The liquid is thoroughly cooled and allowed to stand for two hours. It is then filtered on to a smooth filter, and the crystals of uric acid washed twice with cold water, then with alcohol, till the washings are no longer acid, dried at 100° C. (=212° F.), and weighed. To the weight of

uric acid thus obtained 0·001 gramme should be added for every 15 c.c. (= ½ oz.) of mother-liquor, the bulk of which need never exceed 30 c.c.; but no correction need be made for the insignificant trace of uric acid dissolved by the aqueous and alcoholic washings. The uric acid thus isolated is usually only slightly coloured and is practically pure. When derived from highly pigmented urines, the uric acid may retain so much colouring matter as to suggest the presence of an amount of impurity sufficient to vitiate the result. In such case, after washing the precipitate of acid ammonium urate off the filter, rectified spirit equal in bulk to the water present should be added, and, after adding hydrochloric acid, the beaker should be covered and heated for some time on the water-bath.

Instead of weighing the uric acid isolated in the foregoing manner, it may, if preferred, be dissolved in a little hot solution of sodium carbonate, and the liquid treated by process 3 or 4.

2. The precipitate of acid ammonium urate having a perfectly definite composition, it may be titrated with standard alkali and an indicator giving no reaction with uric acid. Such an indicator exists in methyl-orange. The precipitate from 200 c.c. of urine is treated with a known measure, *e.g.*, 20 c.c., of decinormal hydrochloric or sulphuric acid, the liquid boiled for some minutes, cooled, diluted to 200 c.c., a few drops of a 1 per cent. aqueous solution of methyl-orange added, and $\frac{1}{20}$-normal caustic soda (= 2·0 grammes of NaHO per litre) dropped in from a burette until the orange colour of the acid liquid becomes yellow, which change indicates the point of neutrality. The difference between the volume of acid employed and that of the alkali required to neutralise it represents the ammonia of the precipitate, uric acid having no action on methyl-orange. Each centimetre of $\frac{N}{20}$

solution of soda shows the presence of 0·0084 gramme of uric acid.

3. An alternative plan, when the acid urate of ammonium is not very strongly coloured, is to rinse the precipitate off the filter with hot water, cool the solution, and dilute it with distilled water to 100 c.c. Twenty c.c. of pure concentrated sulphuric acid is then added, so as to acidify the liquid and raise its temperature to about 60° C. (= 140° F.), and then a standard solution of potassium permanganate is run in, till the liquid acquires a pink tint surviving agitation and lasting some seconds. Further decolorisation may occur on standing, but this should be disregarded. Each centimetre of $\frac{1}{20}$ normal permanganate (= 1·578 gramme of $KMnO_4$ per litre) decolorised represents 0·00375 gramme of uric acid.[1] F. G. Hopkins strongly recommends this process (*Jour. Pathology and Bacteriology*, June 1893). When it is intended to titrate the ammonium urate with standard permanganate in the above manner, it is very desirable to wash the precipitate with a saturated solution of ammonium sulphate, instead of ammonium chloride, since the latter salt somewhat affects the accuracy of the titration.

The same method of titration by permanganate may be applied to the uric acid isolated in process 1, after simply dissolving it in a little hot solution of sodium carbonate.

4. Bayrac (*Compt. rend.*, cx. 352) determines uric acid by evaporating 50 c.c. of the urine to dryness at 100° C., treating the residue with 10 c.c. of dilute hydrochloric acid (1 : 5), and washing with alcohol to

[1] This factor is due to F. G. Hopkins, as the result of experiment. As it corresponds to no simple reaction, the process has been investigated in the author's laboratory by A. R. Tankard. The first results on pure uric acid were not very constant, ranging from 96 to 104 per cent. of the truth, but on taking as the end-reaction the point at which the permanganate ceased to be *instantly* decolorised much closer figures were obtained. When the titration was conducted at a boiling heat, instead of at 60° C., the results were higher.

remove urea and creatinine. The residue is dissolved on the water-bath in 20 drops of caustic soda solution, and heated to 90° or 100° C. with 15 c.c. of a concentrated solution of sodium hypobromite solution in the apparatus for estimating urea shown in fig. 11, page 145. 22·38 c.c. of nitrogen measured at 0° C. and 760 mm. barometric pressure, or 23·55 c.c. at the ordinary temperature and pressure, are said to represent 0·084 gramme of uric acid.

Experiments made in the author's laboratory to test the possibility of estimating uric acid by measuring the amount of nitrogen evolved on treatment with alkaline hypobromite have not yielded very encouraging results, the reaction being subject to variations from causes not yet understood.

5. Uric acid can be determined by heating it with concentrated sulphuric acid, and determining the ammonia by treatment with hypobromite or distillation with alkali. The method can be applied to a uric acid precipitate. (See page 127 *et seq.*)

F. B. Haycraft has described a method of determining uric acid based on its precipitation as silver urate, on adding a solution of ammonio-nitrate of silver. The process has been modified by Salkowski and others. It is somewhat complex, and the results are not more accurate than those yielded by methods already detailed.

F. W. Pavy (*Trans. Royal Med. and Chirurg. Soc., London*) has proposed to determine uric acid volumetrically by an ammoniacal cupric solution, in the manner so successfully employed for glucose (page 67); but the reducing power of uric acid on Pavy's solution is found in practice to vary so greatly with the method of conducting the titration and other working conditions as to render the results of very little value.

Urates.

Uric acid is a feeble acid which is usually stated to possess a dibasic function. But it was shown by Bence Jones (*Jour. Chem. Soc.*, xv. 201), and has been confirmed more recently by Sir Wm. Roberts (*Croonian Lectures*, 1892), that a third series of urates exist and have great physiological significance. The salts of the formula $M_2C_5H_2N_4O_3$, commonly called *neutral* or *normal urates*, dissolve readily in water, and are exclusively laboratory-products, not being met with in the animal system under either healthy or pathological conditions. The *acid urates*, or "bi-urates," of the formula $MHC_5H_2N_4O_3$ are very sparingly soluble, and exist in the urine only after it has undergone ammoniacal fermentation. They are known pathologically as components of gouty concretions in the tissues, but it is questionable if they ever exist physiologically in the blood or tissues. The third class, or "quadri-urates," have the composition $MHC_5H_2N_4O_3,H_2C_5H_2N_4O_3$. They are more soluble than the bi-urates, and are specially the physiological combinations of uric acid. They exist normally in the urine, and probably also in the blood, and constitute the whole of the urinary excretion of birds and serpents. Roberts considers that all the morbid phenomena due to uric acid probably arise from secondary changes in the quadri-urates.

QUADRI-URATES, $MHUr,H_2Ur$, usually present themselves as amorphous powders, but the spheres of birds' and serpents' urine are distinctly crystalline, and display a black cross when examined by polarised light. These forms are permanent in the air if kept perfectly dry, but readily assume a gelatinous character, and then appear under the microscope as large translucent globules. The quadri-urates are difficult to obtain pure. When produced artificially, they are apt to be mixed

with free uric acid or bi-urates, and when prepared from urine to be contaminated with pigments and traces of extraneous saline matters. Roberts prepares potassium quadri-urate by adding 2 grammes of uric acid to a boiling solution of 9 grammes of potassium acetate in 300 c.c. of water. The liquid is agitated for about a minute, filtered hot, and cooled rapidly in a stream of cold water. The voluminous precipitate which forms is filtered off, washed in succession with rectified spirit and absolute alcohol, and dried at a temperature not exceeding 40° C. The results of the analysis of the product obtained agree well with the formula $KHC_5H_2N_4O_3,H_2C_5H_2N_4O_3$. Other quadri-urates can be obtained by similar means, but they are less stable than the potassium salt.

The quadri-urates are insoluble in alcohol, ether, chloroform, glycerin, and volatile oils, and cannot be dissolved without change in any simple menstruum. When treated with hot water, they pass momentarily into solution, but are almost immediately decomposed into bi-urate and free uric acid. The same decomposition is effected by neutral saline solutions, but in this case, and notably with a solution of common salt, the decomposition is greatly retarded. When treated with solutions of alkaline carbonates, or disodium hydrogen phosphate, the quadri-urates are converted into bi-urates. In healthy urine of feeble acid reaction the quadri-urates dissolve unchanged, but the solution undergoes gradual but complete decomposition, with ultimate separation of the whole of the uric acid in a free state. This change is retarded in normal urine by the salts and colouring matters present (urea has no influence), occurring with greater facility the larger the proportion of free acid there is present.

In studying the action of water on quadri-urates Sir W. Roberts recommends that about 0·4 gramme of

the dried deposit should be stirred up with 1000 parts (= 400 c.c.) of distilled water, the mixture heated nearly to the boiling point until solution is complete, and then left at rest for forty-eight hours. The supernatant liquid is then syphoned off and the remainder passed through a weighed filter. The crystals of uric acid are washed very sparingly with cold water, then more freely with rectified spirit, dried, and weighed.[1] A correction of 0·0055 gramme per 100 c.c. of mother liquor is applied to compensate for the solubility of the uric acid. The decanted liquid, filtrate, and washings are next heated nearly to boiling, strongly acidulated with hydrochloric acid, and allowed to stand forty-eight hours as before.

By the foregoing method, Sir W. Roberts obtained the following figures from two specimens of quadri-urate prepared by the acetate of potassium method.

	Sample A.	*Sample B.*
Uric acid separated by water (corrected for solubility),	0·080 gramme.	0·164 gramme.
Uric acid dissolved as bi-urate,	0·077 „	0·159 „

These results sufficiently establish the existence of the quadri-urate and the manner of its decomposition by water.

The decomposition of sodium quadri-urate under the influence of water can be conveniently observed by filtering off the buff-coloured sediment deposited by healthy urine, washing it thoroughly with cold rectified spirit, and drying it at a blood-heat. When the quadri-urate thus purified is mixed with a considerable volume of water it is speedily disintegrated, a portion passing into solution in combination with the bases, and the remainder falling as an insoluble precipitate of crystalline uric acid. The change is readily observed under the microscope by intimately mixing a particle

[1] It is evident that the modified methods of determining uric acid, described on page 168 *et seq.*, may with advantage be employed here.

of the purified deposit on a glass slide with a drop of water and protecting the mixture with a covering-glass. In the course of ten minutes ovoid crystals of uric acid make their appearance, and grow and multiply till in the course of half an hour the entire field is thickly studded with crystals; the process continuing, provided that water be added as required, until the amorphous substance is entirely replaced by crystals of uric acid.[1]

The quadri-urates readily assume a gelatinous form. Thus, if a 5 per cent. solution of ordinary sodium phosphate be heated to boiling with excess of uric acid, and the liquid filtered hot, the filtrate sets to a jelly on cooling. This jelly, after being pressed between blotting-paper to free it from mother liquor, exhibits the characteristic behaviour of a quadri-urate, being rapidly decomposed by water with copious formation of crystals of uric acid. On keeping in a moist condition, gelatinous sodium quadri-urate gradually passes into a crystalline condition, and then appears under the microscope in radiating spheres, exactly similar to the spheres so common in serpents' and birds' urine.

If the white mortar-like substance which constitutes the urinary excretion of birds and serpents be examined in its fresh and uncontaminated state, and not after contact with water or bacterial fermentation, it will be found to behave in an exactly similar manner to artificial quadri-urates. Under the microscope it appears as minute spheres, which exhibit a radiated structure and display a black cross with polarised light. On adding a drop of water, the spheres are seen gradually to melt away, with formation of hexagonal tablets of uric acid.

BI-URATES have the general formula $MHC_5H_2N_4O_3$,

[1] Crystals of the sodium bi-urate simultaneously formed are never observed, since this salt is liberated in the gelatinous form.

or MHUr. They result from the action of water on the quadri-urates, and exist in the body under various pathological conditions. The sodium salt, which is the most important and characteristic member of the series, possesses the following properties:—

Acid Sodium Urate, or *Sodium Bi-urate*, contains $2(NaHC_5H_2N_4O_3) + H_2O$. It generally forms a crystalline powder, which, under the microscope, appears in needles (often crossed), rosettes, stellate, and hedgehog-like forms. It requires about 1200 parts of cold or 120 of boiling water for solution. Sodium bi-urate is readily obtained by passing carbon di-oxide through a solution of uric acid in caustic soda, or by boiling uric acid with sodium carbonate, phosphate, or acetate, or with borax. The buff or brick-red sediment often thrown down by urine is commonly stated to consist of sodium bi-urate, but Roberts has shown that it consists essentially of sodium quadri-urate (page 175).

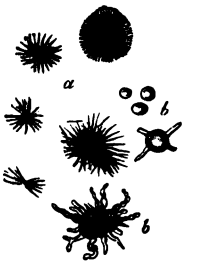


Fig. 17. — ACID SODIUM URATE. (After Frey).

The solubility of acid urate of sodium in water impregnated with salt and other substances has an important bearing on the cause and cure of gout, and has been investigated by Sir Wm. Roberts, who gives the figures on next page, which represent the parts by weight of sodium urate dissolved at 100° F. (= 37·8° C.) by 1000 parts of the solution of the strengths indicated. The amount of sodium bi-urate dissolved by 1000 parts of distilled water at 100° F. was found to be 1·0. From the following results it appears that the solvent action of the various salts depends on the nature of the metal, and has no reference to its form of combination. Salts having an alkaline reaction to litmus, like the carbonates and phosphates, behave exactly similarly to

those of neutral reaction, such as the chlorides and sulphates. Salts of potassium exert no appreciable influence on the solubility of sodium bi-urate in water. Salts of sodium decrease the solubility, the influence being greater the larger the proportion of salt present. Salts of ammonium, calcium, and magnesium behave similarly to, but less powerfully than, salts of sodium.

Percentage of Salt in Solvent.	0·1 per cent.	0·2 per cent.	0·3 per cent.	0·5 per cent.	0·7 per cent.	1·0 per cent.
Sodium bicarbonate,	0·50	0·34	0·20	0·13	0·09	0·08
Sodium chloride,	0·45	0·30	0·16	0·10	0·08	0·05
Sodium phosphate (crystallised),	0·70	...	...	0·32	...	...
Sodium sulphate (crystallised),	0·55	...	...	0·24	...	...
Sodium salicylate,	0·65	...	0·36	0·25	...	...
Potassium bicarbonate,	0·96	1·00	1·00	0·97	1·02	0·98
Potassium chloride,	0·96	...	1·01	1·10	...	...
Potassium phosphate,	1·01	...	...	1·00	...	...
Ammonium chloride,	0·85	0·50	0·42	0·35	...	...
Calcium chloride,	...	...	...	0·27	...	...
Calcium sulphate,	0·65	0·44	...	...	...	...
Magnesium chloride,	0·85	...	...	0·68	...	...
Magnesium sulphate (crystallised),	0·90	...	...	...	...	...

Crystalline sodium bi-urate is ten times as soluble in boiling water as in cold, but a saturated hot solution does not deposit the excess of salt immediately on cooling. The bi-urate remains in complete solution for a considerable time, and is not entirely deposited for some days. Roberts has shown that this behaviour is not merely due to supersaturation of the liquid, but is owing to the formation of a gelatinous modification of the bi-urate of greater solubility than the crystalline form. Thus if a saturated solution of sodium bi-urate in boiling water be prepared, and when cold mixed with an equal measure of a 20 per cent. solution of common salt, a voluminous gelatinous precipitate will be thrown down. Saturated solutions or solid crystals of other salts (*e.g.*, sodium phosphate or acetate, potassium chloride, phosphate, acetate, &c.) may be substituted for the common salt. The precipitate, if filtered off,

allowed to drain, and cautiously washed with cold water, consists of sodium bi-urate in a state of approximate purity. It dissolves at 100° F. in blood-serum, or in a liquid containing 0·5 gramme of sodium chloride and 0·2 of sodium carbonate per 100 c.c. (which represents the saline ingredients of serum), sufficiently freely to cause a considerable separation of uric acid after acidulating with acetic acid; whereas crystalline sodium bi-urate is taken up by water at 100° F. so slightly that no deposition of uric acid is obtainable on acidulating the liquid.

The gelatinous form of sodium bi-urate gradually changes into the crystalline variety, and the gradual deposition of the salt from its solution in water, blood-serum, or imitation-serum is evidently due to the same change of condition.

Acid Potassium Urate, or *Potassium Bi-urate*, KHUr, is said to be sometimes formed as a urinary deposit in cases of fever. It is amorphous, and more soluble than the corresponding sodium salt, requiring for solution only 800 parts of cold or 70 to 80 parts of boiling water.

Acid Lithium Urate or *Lithium Bi-urate*, LiHUr, forms crystalline grains, soluble in 370 parts of cold or 39 of boiling water. Lipowitz states that if equal parts of uric acid and lithium carbonate be treated with 90 parts of water at blood-heat, a clear solution is obtained, while at 100° C. four times the amount of uric acid can be dissolved without increasing the weight of lithium carbonate. Seeing that lithium carbonate itself requires about 200 parts of water for solution, its solvent action on uric acid is remarkable, and is of much interest in connection with the extensive application of lithium salts in the treatment of gout. On the other hand, it is stated by L. Siebold (*Year-book Pharm.*, 1889, page 413), as

the result of direct experiment, that the relative solvent action of solutions of lithium, sodium, and potassium carbonates on a given weight of uric acid, under equal conditions of dilution and at a temperature of 37° C. (blood-heat), is strictly proportional to the ratio of the molecular weight of these solvents. Hence lithium carbonate has the advantage that 74 parts are chemically equivalent to 106 of the sodium salt or 138 of potassium carbonate; but there the advantage ceases. Urinary sediments are similarly dissolved by these carbonates with equal facility if molecular proportions are used, and equivalent weights of the citrates of lithium, sodium, and potassium produce equal alkalinity in the urine of the person taking them. Siebold further states that lithium chloride and sulphate have no solvent action on uric acid and acid urates, and that natural mineral waters containing these salts have none beyond that exercised by basic constituents simultaneously present, and by the water.

Acid Ammonium Urate, $(NH_4)HUr$, is soluble in about 1500 parts of cold water, and quite insoluble in saturated solutions of ammonium chloride and sulphate (compare page 168). The urinary excrement of serpents is commonly stated to consist almost wholly of a mixture of acid urate of ammonium with free uric acid. This is often true of the altered product, but Sir W. Roberts has shown that, in a fresh and undecomposed state, serpents' urine consists substantially of quadri-urates, which undergo decomposition into a mixture of bi-urates and free uric acid by contact with water (page 176). Guano, the excrement of various aquatic birds, consists chiefly of oxalate and acid urate of ammonium in admixture with phosphates. Guanine, $C_5H_5N_5O$, a base forming crystallisable salts with acids, is also a constituent of guano, and

replaces uric acid in the urine of spiders and other invertebrate animals.

NEUTRAL or NORMAL URATES of the light metals do not exist naturally, but they may be obtained by dissolving uric acid in the theoretical amount of alkali. The normal urates of lithium and ammonium are unknown. *Neutral Potassium Urate*, $K_2C_5H_2N_4O_3$, forms small crystals having an alkaline reaction and caustic taste. It dissolves, with partial decomposition into the acid salt, in about 36 parts of cold water, forming a liquid of soapy taste which froths strongly when shaken. *Normal Sodium Urate*, $Na_2Ur + H_2O$, forms hard nodules which closely resemble the potassium salt, but are less soluble in water.

On passing carbon dioxide through a solution of the normal urate of potassium or sodium the corresponding acid urate is precipitated. The same decomposition occurs by prolonged boiling of the solution.

The following table, due to Ralph (*Practical Treatise on Diseases of the Kidneys*, 1885), shows the characters of the urates of the light metals:—

Urate.	Solubility in Cold Water.	Character of Deposit.
Potassium; acid, .	1–800	Amorphous.
,, normal,	1–44	Amorphous, or in fine needles.
Sodium; acid, .	1–1200	Amorphous; rarely crystalline.
,, normal,	1–77	Nodular masses.
Lithium; acid, .	1–60	Amorphous, or in fine needles.
Calcium; acid, .	1–600	Amorphous, or in fine needles.
,, normal,	1–1500	Fine granules.
Ammonium; acid,	1–1600	Amorphous, or spiked globular masses.

The urates of lead, copper, mercury, and silver are quite insoluble in water. Hence solutions of these metals are used for determining uric acid or for separating urates from urine.

The behaviour of the urates with water and saline solutions has an important bearing on the cause and treatment of gout. It is probable that in media containing alkaline carbonates—such as the serum of the blood, and its derivatives lymph and synovia—uric acid passes into solution in the first instance as quadri-urate, and it may be inferred that it circulates in the blood and is voided in the urine in the same form. In perfect health, the elimination of the quadri-urate proceeds with sufficient speed and completeness to prevent any undue detention or any accumulation of it in the blood. But in gouty subjects, either from defective action of the kidneys or from excessive introduction of uric acid into the circulation, the quadri-urate lingers unduly in the blood and accumulates therein. The detained quadri-urate, circulating in a medium rich in carbonate of sodium, gradually takes up an additional atom of base, and is thereby converted into bi-urate, which at first exists in the hydrated or gelatinous condition, but with lapse of time and accumulation passes into the insoluble crystalline condition, and then symptoms of gout manifest themselves.

A. Haig (*Med. Chirurg. Trans.*, lxxi. 125, 283) has shown that administration of acids diminishes the relative amount of uric acid excreted, while that of alkalies increases it. Thus the normal proportion of uric acid to urea is 1 : 35, but after a few doses of citric acid the relation was 1 : 41, and after similar doses of potassium citrate 1 : 28. In these cases there was not only a relative but also an absolute diminution and increase in the uric acid excreted. Salicylic acid forms an important exception to the general behaviour of acids, for while it increases urinary activity it does not in any way diminish the excretion of uric acid. Moreover, acids given while

salicylates are present in the circulation have no longer the power of diminishing the excretion of uric acid, nor is excessive excretion of uric acid under salicylates accompanied by any headache. Both uric and salicyluric acids are present in the urine passed under the influence of salicylates, probably owing to the salicylate acting on the uric acid in the blood, but not on that secreted by the kidney itself. Benzoates do not act in the same way as salicylates, probably because the hippuric acid formed from them is less soluble than salicyluric acid. The value of salicylates in uric acid diseases is largely due to their power of preventing acids from causing retention of uric acid. Thus salicylates prevent gout, the peculiar headache due to uric acid and frequent after breakfast, and also epilepsy, which is probably due to uric acid acting on the nerve-centres.

Hippuric Acid. Benzoyl-amidoacetic Acid. Benzoyl-glycocine. $C_9H_9NO_3$; or, $C_6H_5.CO.NH.CH_2.COOH$.

Hippuric acid derives its interest less from its pathological importance than as affording a typical example of the so-called "conjugated bodies" of which the synthesis is readily effected within the living organism. Thus if benzoic acid be taken internally, it appears in the urine as hippuric acid, and hippuric acid may be obtained artificially by heating benzoic anhydride with amido-acetic acid (glycocine), or the zinc salt of the latter with benzoyl chloride :—

$$C_7H_5O.Cl + C_2H_2(NH_2)O.OH = C_7H_5O.O.C_2H_2(NH_2)O + HCl$$

Benzoyl Chloride. Glycocine. Hippuric Acid.

Benzoic aldehyde, toluene, cinnamic acid, quinic acid and phenyl-propionic acid when ingested, are also excreted as hippuric acid. Substituted benzoic acids appear in the urine as substituted hippuric acids. Salicylic acid, which has the constitution of

ortho-hydroxybenzoic acid, $C_6H_4(OH).COOH$, is converted in the system into hydroxy-hippuric or salicyluric acid, $C_9H_8(OH)NO_3$, which may be detected in the urine by the bluish-violet coloration produced on adding dilute ferric chloride.

The quantity of hippuric acid excreted in normal human urine is stated to range from 5 to 60 grains (= 0·3 to 3·8 grammes) in twenty-four hours, but an increase results from a vegetable diet. This has been particularly noticed after eating plums, pears and cranberries, and the cuticular parts of many plants act similarly. In the urine of diabetic patients, hippuric acid is frequently present in much increased proportion, as also in jaundice and other liver-complaints, and it is abundant in the acid urine of persons suffering from all kinds of fevers.

Hippuric acid replaces uric acid in the urine of herbivorous animals, which are stated to contain it to the extent of about 2 per cent.;[1] its origin being doubtless in bodies of the aromatic series existent in the food. Hippuric acid is also found in the excrement of the lower animals, except that of birds, which contains the allied substance ornithuric acid, having the constitution of a dibenzoyl-diamidovaleric acid:[2]—

$$\left.\begin{matrix} C_6H_5.CO.NH \\ C_6H_5.CO.NH \end{matrix}\right\} : C_4H_7.COOH.$$

[1] Hippuric acid may be conveniently prepared from *fresh* cows' or horses' urine, which often contains sufficient to yield a precipitate on mere addition of excess of hydrochloric acid. If not, the urine should be boiled with milk of lime, and the filtrate neutralised, concentrated, and treated with excess of hydrochloric acid; or the neutralised filtrate may be precipitated with ferric chloride, and the washed precipitate decomposed by hydrochloric acid. The hippuric acid is freed from colouring matter by recrystallising it from chlorine-water, or by treating it with bleaching powder and hydrochloric acid.

[2] On boiling ornithuric acid with hydrochloric acid, it almost immediately parts with one benzoyl group and yields benzoyl-ornithine, which on further boiling splits up into benzoic acid and diamido-valeric acid or ornithine, $(NH_2)_2C_4H_7.COOH$, a base of strong alkaline reaction and of caustic taste.

Hippuric acid crystallises in milk-white rhombic prisms ending in two or four facets, the crystals being often grouped in clumps (fig. 18). As liberated by adding hydrochloric acid to cows' urine or one of its salts, hippuric acid is apt to form ill-defined crystals.

Fig. 18. — HIPPURIC ACID (after Frey). *a*, *a*, Prisms; *b*, Crystals formed by slow evaporation.

Hippuric acid has a slightly bitter taste, free from acidity. It melts at 187·5° C., and above 240° decomposes, with an odour of hay or fresh urine, and formation of hydrocyanic and benzoic acids and benzonitril (phenyl cyanide), $C_6H_5.CN$, a dark resinous or coaly mass being left.

Hippuric acid requires about 600 parts of ice-cold water for solution, but dissolves tolerably readily in hot water. It is also soluble in alcohol, especially when hot. The aqueous and alcoholic solutions have an acid reaction. Hippuric acid is but slightly soluble in cold ether, but dissolves in acetic ether, and readily in boiling amylic alcohol. In chloroform, benzene, petroleum spirit, and carbon disulphide it is practically insoluble.

When boiled for a time (half an hour) with dilute nitric, hydrochloric, or oxalic acid (or more rapidly if strong hydrochloric acid be used), hippuric acid undergoes hydrolysis, the liquid on cooling depositing benzoic acid, while a salt of glycocine (amido-acetic acid) remains in solution :— $C_9H_9NO_3 + H_2O = C_7H_6O_2 + C_2H_5NO_2$. A similar reaction takes place spontaneously in urine containing hippuric acid, under the influence of ferments. Hence only perfectly fresh urine will yield hippuric acid. If the urine be

alkaline, as is usually the case with that of herbivorous mammals, the glycocine first produced splits up into ammonia and acetic acid :—$C_2H_5NO_2+H_2O=C_2H_4O_2+NH_3$.

If hippuric acid be evaporated to dryness with concentrated nitric acid, and the residue heated, an odour of nitrobenzene is evolved.

Hippuric acid decomposes carbonates, and dissolves zinc with evolution of hydrogen. Its salts are mostly soluble and crystallisable. The hippurates of silver, lead, and copper are sparingly soluble.

When ferric chloride is added to a solution of a hippurate, a cream-coloured precipitate of ferric hippurate is thrown down, which contains more or less basic salt, according to the greater or less dilution of the solution. The precipitate is almost insoluble in pure water, but dissolves in free hippuric acid, in excess of ferric chloride, and in alcohol. The reaction with ferric salts may be employed for the determination of hippuric acid in urine. For this purpose, the urine is acidulated with nitric acid, heated to boiling to remove carbon dioxide, neutralised with calcium carbonate, treated with excess of lead nitrate, and then diluted to a known volume and filtered. An aliquot part of the filtrate is then heated and titrated with a solution of neutral ferric nitrate which has been standardised with pure hippuric acid. The reaction is at an end when a drop of the clear liquid gives a blue coloration with potassium ferrocyanide. The distinction between this point and the previous formation of the white ferrocyanide of lead is very sharp.

On treating ferric hippurate or the solution of a soluble hippurate with excess of hydrochloric acid, the hippuric acid separates sooner or later in long crystalline needles. Hippuric acid is distinguished from

benzoic and salicylic acids by its crystalline form (figs. 18, 19, pages 185, 187), by charring when heated with strong sulphuric acid, by giving off ammonia on ignition with soda-lime, and by not being dissolved on agitating its solution with chloroform or petroleum-spirit.

For the detection of hippuric acid in the urine of herbivora, the fresh liquid should be treated with milk of lime, filtered, the filtrate concentrated to a syrup as rapidly as possible, and excess of hydrochloric acid added, when hippuric acid crystallises out on standing. If the urine be at all stale, benzoic acid will be obtained instead, and may be distinguished from hippuric acid as just described.

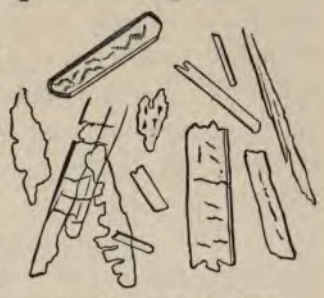


Fig. 19.—BENZOIC ACID.

For the detection of traces of hippuric acid in human urine, the perfectly fresh liquid should be evaporated nearly to dryness at 100° C., the residue mixed with powdered barium sulphate, a little hydrochloric acid added, and the whole exhausted with rectified spirit. The alcoholic solution is carefully neutralised with soda, and the liquid evaporated. The residue is mixed with a little oxalic acid, and again evaporated to dryness on the water-bath. The residue is exhausted with a mixture of equal measures of alcohol and ether, the solution distilled to a small bulk, boiled with milk of lime, filtered, concentrated, and acidulated with hydrochloric acid. Immediately, or on standing, according to the quantity present, crystalline needles of hippuric acid separate, and may be filtered off and purified by washing, first with diluted hydrochloric acid and then with a little ether.

Another method is to precipitate the urine (1000 to

1200 c.c.) with a slight excess of strong baryta-water. The filtered liquid is treated with dilute sulphuric acid till exactly neutral to litmus, decanted or filtered from the precipitated barium sulphate, and evaporated to a syrup on the water-bath. The residue, which should be exactly neutral, is treated while still hot with 150 to 200 c.c. of absolute alcohol and thoroughly agitated. Barium succinate, sodium chloride, and other compounds are thus precipitated. The liquid is decanted, the alcohol evaporated, and the syrupy residue treated while still hot with hydrochloric acid. The liberated hippuric acid is extracted by repeated agitations with ether (100 to 150 c.c.), the separated ether distilled off, the residue diluted with water, and heated to boiling with a little milk of lime. The liquid is filtered, concentrated, and treated with excess of hydrochloric acid, when hippuric acid separates in fine crystals, which can be obtained colourless by treatment with purified animal charcoal.

COLOURING MATTERS OF URINE.

THE colour of urine is liable to modification under the influence of a variety of pathological conditions. The following *résumé* is based on that of Krukenberg:—

Nearly colourless or pale yellow urine may be due either to greater dilution or to diminution of the normal pigments. It occurs specially in anæmia, chlorosis, diabetes, granular kidney, &c.

Yellowish and milky urine may be due to the presence of floating globules of fat, as in chyluria; or to suspended pus-corpuscles, as in pyelitis or other purulent disease of the urinary tract.

Orange-coloured urine is excreted after the ingestion of certain drugs, such as chrysophanic acid, rhubarb, santonin, &c.[1]

Brownish-yellow to red-brown urine, which becomes blood-red on addition of an alkali, is likewise indicative of excreted drugs, such as rhubarb, senna, chelidonium, &c. Port-wine coloured urine is sometimes excreted after sulphonal has been taken.

Dark yellow to brown-red urine, which easily deposits a sediment, occurs in certain febrile diseases, and owes its colour to an increase of the normal pigments or to the occurrence of pathological colouring matters.

Red or reddish urine may be due to pigmentary

[1] If the urine be treated with caustic alkali and then shaken with amylic alcohol, the colouring matter of santonin is dissolved out, and becomes yellow on exposure to air. The colouring matter of rhubarb (chrysophanic acid) is not sensibly dissolved by amylic alcohol.

matters (*e.g.*, logwood, madder, bilberries, coal-tar dyes) in the food, or to the presence of unchanged hæmoglobin, occurring in hæmoglobinuria or in urinary hæmorrhage. A *brown* colour may also be due to hæmorrhage into the kidneys or to methæmoglobinuria.

Brown or brownish-black urine is excreted in cases of methæmoglobinuria and hæmorrhage, and is especially indicative of melanitic sarcoma. It is also highly characteristic of poisoning by carbolic acid, in which case hydrochinone and catechol (pyrocatechin) appear in the urine.[1]

Yellowish-green, green, or greenish-brown urine is indicative of the presence of bile-pigments, and of course it occurs in jaundice and other affections of the liver.

Dirty green or blue urine, sometimes showing a dark *blue scum* with a *blue deposit*, indicates the presence of excess of indigogens. It frequently occurs in cholera and typhus,[2] especially when the urine is putrefying.

The chemistry of urochromes is still in a very confused state, notwithstanding the great attention the subject has received. An exhaustive recapitulation of

[1] Melanitic urine, on addition of ferric chloride, yields a brown turbidity or black precipitate soluble in excess. On adding a dilute solution of sodium nitroprusside, followed by caustic alkali, they frequently give a pink or red coloration, and on adding an acid Prussian blue is precipitated.

[2] The urine of healthy persons gives a negative reaction with the "diazo test," while, on the other hand, the urine of patients suffering from typhus and certain other fevers exhibits the reaction in a marked manner. The reaction is also produced in cases of acute tuberculosis, but not by the urine in intestinal catarrh, which can thus be differentiated from typhus fever. (See Rutimeyer, *Lancet*, ii., 1890, page 413.) To apply the diazo test, 1 gramme of sulphanilic acid is dissolved in 10 c.c. of pure hydrochloric acid, and the solution diluted with water to 200 c.c. Fifty c.c. of this reagent is mixed with 5 c.c. of a solution of 1 gramme of potassium or sodium nitrite ($NaNO_2$) in 200 c.c. of water, and 50 c.c. of the sample of urine added. The mixture is made strongly alkaline by ammonia, and the whole well shaken. A bright red colour is produced by the pathological urines above mentioned, and on standing for twenty-four hours a deposit forms, the upper part of which is green or black. Healthy urine gives no such reaction.

the existing knowledge on the subject is beyond the scope of this work, but the following is a brief outline of the leading facts known respecting the principal colouring matters of normal and pathological urine.

Urobilin.

The best known and most definite of the colouring matters of normal urine is *urobilin*, to which the formula $C_{32}H_{40}N_4O_7$ or $2C_{16}H_{18}N_2O_3+H_2O$ is ascribed. It is a yellowish-brown amorphous substance, almost insoluble in pure water but soluble in presence of small quantities of free acids or neutral salts, slightly soluble in ether and benzene, and readily so in alcohol and chloroform. The neutral alcoholic solution is reddish-yellow, and when concentrated exhibits a green fluorescence. On addition of acid, the fluorescence is destroyed, and the dilute solution acquires a rose tint. Alkaline solutions of urobilin are yellowish or yellowish-green, according to their concentration, and show a fluorescence which is much more marked after addition of a solution of zinc chloride, the liquid then appearing rose-coloured by transmitted and green by reflected light. Neutral and alkaline solutions of urobilin in alcohol, when examined in the spectroscope, exhibit an absorption-band between the Fraunhöfer-lines *b* and F. The absorption is much increased by the addition of zinc chloride. Normal urobilin is regarded by MacMunn as an oxidation-product of effete hæmatin and bile-pigments and not as a reduction-product. It appears to be identical with *choletelin*, a body resulting from the treatment of hæmatin in acid solution with hydrogen peroxide, and said by Maly to have the formula $C_{16}H_{18}N_2O_6$.

The proportion of urobilin in fresh normal urine is often extremely small, but appears to increase on

exposure of the urine to the air. This behaviour is probably due to the presence in the urine of a chromogen or mother-substance, which is converted into urobilin by oxidation.

For the extraction and estimation of urobilin, A. Studensky (*Chem. Centr.*, 1893, ii. 668) recommends that 20 c.c. of the urine should be treated with 2 c.c. of a saturated solution of cupric sulphate, the liquid saturated with crystallised ammonium sulphate, 10 c.c. of chloroform added, and the mixture shaken for some minutes. The urobilin dissolves in the chloroform with copper-red colour, while bilirubin and its allies are not extracted in presence of copper sulphate. A portion of the chloroformic layer is tapped off, and its colour compared with that of a standard solution of urobilin, prepared in a similar manner from a large quantity of urine. The chloroformic solution thus obtained is evaporated to dryness, the residue washed with ether, and weighed. Standard solutions of urobilin are prepared by dissolving known weights of this residue in chloroform, and the solution yielded by the sample under examination is compared with them. The standard solutions remain unchanged for some months, if preserved in the dark in closed vessels, and covered with a layer of saturated solution of ammonium sulphate.

Pathological Urobilin is, according to MacMunn, a substance distinct from normal urobilin, though obtainable in the same manner and soluble in the same menstrua as that body. The absorption-band at F, which characterises the spectrum of the urobilin series, is much broader and darker in the case of the pathological than in that of the normal colouring matter, and other characteristic differences are recognised by MacMunn, who regards the former as less oxidised than the normal product and indistinguish-

able from and probably identical with stercobilin, the colouring matter of fæces.[1]

UROERYTHRIN is the colouring matter of certain bright pink urinary deposits, and possibly of the highly coloured urine excreted in rheumatism. It may be extracted by boiling pink urates with alcohol. The solution exhibits two ill-defined absorption-bands between D and F, and the residue left on evaporation is turned green by caustic alkalies.

UROMELANIN is a dark-coloured pigment produced by boiling urine with hydrochloric acid. According to L. von Udransky, it has its origin in the carbohydrates of the urine (*Zeitsch. physiol. Chem.*, xi. 537; xii. 33).

URO-EOSEÏN is the name given to an extremely unstable pigment observed by Nencki and Sieber in diabetic urine which became bright pink on adding hydrochloric acid. It was extracted by amylic alcohol, and showed a characteristic absorption-band between D and E.

Bile Pigments in Urine.

The colouring matters characteristic of bile are not present in normal urine, but in certain diseases (jaundice, &c.) they exist in very appreciable amount. Such urine exhibits a yellowish-green, green, greenish-brown, or almost black colour. The most characteristic colouring matter of bile is *bilirubin*, $C_{16}H_{18}N_2O_3$, while *biliverdin*, *biliprasin*, *bilifuscin*, *bilicyanin*, &c., are

[1] MacMunn points out that, "if this be so, then the presence of pathological urobilin in urine is, to a certain extent, an indication of the absorption of fæcal matters from the intestine, and with them of poisonous alkaloidal bodies—ptomaines, which have escaped the destructive action of the liver. . . . By such an assumption we can explain many of the symptoms which accompany its presence in urine. . . It would appear that, in those cases where pathological urobilin occurs in urine, there is either some vaso-motor disturbance of the hepatic circulation, or some mechanical interference with it, as in passive congestion."

products of its oxidation. In bilious urine of saffron-yellow colour bilirubin predominates, while biliverdin and other oxidation-products are present in greenish urine. Bilious urine gives a yellow froth on agitation, and stains linen and filter-paper yellow.[1] Bilirubin may be extracted from its acidulated solutions by agitation with chloroform. It dissolves in caustic alkalies, and on exposing the solution to the air it is converted into biliverdin, $C_{16}H_{18}N_2O_4$; subsequently into biliprasin, $C_{16}H_{22}N_2O_6$; and, according to Maly, ultimately into choletilin, $C_{16}H_{18}N_2O_6$.

A variety of tests have been proposed for the detection of bile-pigments in urine, but the following are the most delicate and answer every purpose :—

Gmelin's Test consists in treating the urine with strong nitric acid and observing the change of colour produced. The reaction is best observed by allowing some of the urine to run gently onto the surface of some fuming nitric acid contained in a test-tube. If bile-pigments be present, a green ring will become apparent at the point of contact, while below this will appear violet, red, and yellow zones, in the order named. The green colour is alone characteristic of bilious urine, since indigogens give rise to blue and red colorations.

Various modifications of Gmelin's reaction have been proposed, but they possess no advantage over the above mode of applying the test.

Rosin's Test for bile-pigments consists in allowing very dilute iodine solution or bromine-water to flow on to the surface of the urine from a pipette. A grass-green ring is produced at the junction of the two strata.

[1] If the dyed filter-paper be treated with a drop of nitric acid, the margin of the spot will become violet or deep blue, while the centre gradually changes to emerald-green.

For the detection of *traces* of bile-pigments, often of great clinical and physiological importance,[1] the urine should be treated with a moderate excess of lime-water, and the excess of lime precipitated as carbonate, by passing carbon dioxide gas or adding seltzer-water, till the liquid no longer exhibits an alkaline reaction to litmus (or preferably to phenolphthalein). The precipitate is collected on a filter, and treated with fuming nitric acid, when the green and other colours already described will become evident if bile be present. Or the precipitate may be boiled with alcohol acidulated with sulphuric acid, when the supernatant liquid will acquire a grass-green colour, a white deposit of calcium sulphate being simultaneously formed.

Another reliable test for bile-pigments in urine is to treat 30 c.c. (or 1 oz.) of the sample with about one-third of its bulk of a 20 per cent. solution of zinc acetate,[2] after previously neutralising most of the free acid by sodium carbonate. The voluminous precipitate is filtered off, washed, and treated with a little ammonia. In presence of bile-pigments the ammoniacal liquid is usually fluorescent, and either at once or on standing shows the absorption-spectrum of bilicyanin, characterised by bands on each side of the D line, and a third between *b* and F.

[1] In two cases of highly icteric urine, after the occurrence of ammoniacal fermentation Salkowski could detect no bilirubin by Gmelin's test, and extraction yielded no unchanged biliary pigment. He suggests that this decomposition of bilirubin without the formation of any characteristic products was probably the result of the activity of bacteria, and may explain other cases of jaundice, in which the urine, though dark coloured, gave no evidence of the presence of bile-pigments.

[2] Zinc acetate may be readily extemporised by treating lead acetate with zinc sulphate in slight excess, and filtering from the precipitated lead sulphate. Or sodium acetate may be added to a solution of zinc sulphate or chloride, the sodium sulphate or chloride formed simultaneously with the zinc acetate being disregarded.

Blood Pigments in Urine.

Blood occurs in urine in certain diseases. In *Hæmaturia* blood is present, and blood-corpuscles may be recognised by examining the deposit formed on standing under a high microscopic power. In *Hæmoglobinuria*, the urine contains the red colouring matter of the blood, but no actual blood-corpuscles unless blood also be present.

The micro-spectroscope affords the most satisfactory means of detecting blood-pigments in urine. In its absence, the following tests may be applied :—

Heller's Test consists in making the urine strongly alkaline with caustic soda or potash, and heating the liquid to boiling. In presence of blood a bottle-green coloration is produced, and a brownish-red precipitate, consisting of earthy phosphates coloured by blood, is thrown down.

Hæmin Test.—The deposit which separates from the urine on standing is evaporated to dryness at a gentle heat with a small fragment of common salt. The residue is treated with two or three drops of glacial acetic acid, and heated on a glass microscope-slide. On cooling, if blood-pigments were present, microscopic crystals of hæmin as reddish-brown rhomboidal plates will be observed.

According to C. Rosenthal (*Chem. Centr.*, 1886, page 251), Heller's test for hæmoglobin, based on the red coloration produced by warming with aqueous soda, fails when the proportion of blood is less than 1 part per 1000 of the urine. Struve's test, consisting in the isolation of hæmin from the precipitate produced by tannin, is uncertain in its results, but the presence of more than a *minute* trace of iron in the ash resulting from the ignition of this precipitate affords satisfactory evidence of the presence of hæmoglobin in the urine.

UROHÆMATOPORPHORIN is regarded by Mac Munn as solely a reduction-product of hæmatin, which has been produced in the organism by reduction of effete hæmoglobin or effete histo-hæmatin. It can be prepared artificially by the action of sodium-amalgam, zinc and dilute acid, and other reducing agents on hæmatin. In acid solutions, the spectrum exhibits a narrow absorption-band almost coincident with the D line, and another darker band between D and E; besides a feeble shading between these two, and a band at F closely resembling that of urobilin. If the alcoholic solution of the isolated colouring matter be treated with ammonia, the liquid shows a five-banded spectrum closely resembling that of hæmatoporphorin.

HÆMATOPORPHYRIN is a colouring matter produced by the action of strong sulphuric acid on hæmatin or hæmin. According to Nencki and Sieber's first accounts it contains $C_{32}H_{34}N_4O_5$, but in a more recent research (*Monatsh.*, ix. 115) they regard this body as either a mixture or an anhydride of true hæmatoporphyrin, which they prepare by acting on hæmin with a saturated solution of hydrobromic acid gas in glacial acetic acid. To this body they ascribe the formula $C_{16}H_{18}N_2O_3$, identical with that of anhydrous bilirubin, which body hæmatoporphyrin resembles in many of its properties. When introduced into the system, hæmatoporphyrin is partly expelled in the urine, but the greater portion is retained, and probably utilised in the formation of hæmoglobin.

Hæmatoporphyrin is very frequently present in the dark-coloured urine excreted after the administration of sulphonal. O. Hammersten (*Jour. Chem. Soc.*, lxii. 649 and 1136) examined four samples of urine from patients to whom sulphonal had been administered, and found hæmatoporphyrin in each case, but adds that more observations will be

needed before it can be positively stated that sulphonal is the cause of the appearance of hæmatoporphyrin in the excretion.[1] For the detection of hæmatoporphyrin, Hammersten precipitates the urine with barium acetate and filters. The filtrate is precipitated alternately with barium acetate and sodium carbonate until a small filtered portion gives a white precipitate with these reagents. The hæmatoporphyrin is carried down in the precipitate. Both precipitates are washed well and extracted with acidified alcohol. The acid alcoholic solution is diluted with several times its measure of water, and shaken with chloroform, which extracts most of the colouring matter. The chloroformic layer is rapidly tapped off from the upper stratum, washed well with water, and evaporated in shallow basins in the dark. According to Hammersten, the brown residue left after evaporation is soluble with splendid purple colour in chloroform, insoluble in cold water and in very dilute acids, sparingly soluble in cold alcohol, but soluble in hot alcohol, from which it crystallises in needles resembling those of the hæmatoporphyrin hydrochloride of Nencki and Sieber. On spectroscopic examination, the absorption-bands were found to be slightly nearer to the red end of the spectrum than those of the hæmatoporphyrin obtained by Nencki and Sieber. In only one case out of the four did the substance appear to be absolutely identical with their product. In another case the chromogen of a similar colouring matter was met with. A solution of hæmatoporphyrin in ammonia and zinc chloride gives four absorption-bands. The two lying between C and D and between *b* and F disappear within twenty-four hours, the former first. The other two bands are permanent.

[1] Hammersten's suggestion has been fully confirmed by Salkowski and others.

Urinary Indigogens.

Normal urine contains traces of the potassium salt of indoxyl-sulphuric acid, $C_8H_6N.SO_4H$.[1] This body is derived primarily from indole, C_8H_7N, which by oxidation yields indoxyl, $C_8H_5(NH).OH$. By reaction with the elements of sulphuric acid this is converted into an ethereal salt, the potassium compound of which is the substance in question.

Potassium Indoxyl-sulphate, $C_8H_6N.SO_4K$, has received the unfortunate name of "urinary indican," from a supposed identity with plant-indican, the glucoside from which indigo is obtained. The only similarity between the two bodies is that both yield indigo-blue as one of the products of their decomposition.[2]

Potassium indoxyl-sulphate crystallises from hot alcohol in colourless lustrous tables, readily soluble in water but only sparingly in cold alcohol. When boiled with dilute acid it is decomposed into indoxyl and acid potassium sulphate, but is not attacked by alkalies. When the crystals are heated, indigotin (indigo-blue) sublimes, and the same substance is found quantitatively when the acidulated solution is warmed with ferric chloride.

For the detection of indoxyl-sulphuric acid in urine, Jaffe (*Pflüger's Archiv.*, iii. 448) first separates any albumin by boiling the liquid, and treats the filtrate with an equal measure of hydrochloric acid. A dilute solution of bleaching powder is then cautiously added, until the blue colour no longer increases. On

[1] Indoxyl-sulphuric acid is described by some writers as indoxyl-sulphonic acid. The latter name would be applicable to a body of the constitution $C_8H_4(SO_3H)(NH).OH$. This would be isomeric with indoxyl-sulphuric acid, and would not exhibit the readiness of the latter in hydrolysing into sulphuric acid and indoxyl (page 6).

[2] Decomposing urine occasionally forms a bluish-red pellicle, and ultimately deposits microscopic crystals of indigo-blue. A calculus of the same nature has been described.

agitating with chloroform the colouring matter is taken up and can be obtained on evaporation. Jaffe's method is not suitable for the detection of traces of indigogen, as the colouring matter is destroyed by the least excess of the oxidising agent. Hence MacMunn boils the urine with an equal measure of hydrochloric acid and a few drops of nitric acid, cools, and agitates with chloroform. The chloroform is generally coloured violet, and, when examined in the spectroscope, shows two broad absorption-bands, one on either side of the D line. The less refrangible is due to indigo-blue and the more refrangible to indigo-red; though it is doubtful if the latter colouring matter is identical with the indirubin which occurs in commercial indigo.[1]

A. C. Méhu (*Jour. Pharm.*, [5], vii. 122) adds to the urine about 0·5 c.c. of strong sulphuric acid to 1 litre of the sample, and then saturates the liquid with powdered ammonium sulphate, whereby any indigotin or indirubin is precipitated.[2] On treating the precipitate in the cold with proof-spirit the indirubin will be dissolved, while the insoluble indigotin is purified by washing with water, followed by spontaneous drying. Méhu proposes a colorimetric process for the estimation of indigotin, which he dissolves in

[1] For the detection of indirubin, O. Rosenbach (*Jour. Chem. Soc.*, lviii. 1032) adds nitric acid to the boiling urine, cools, adds a large excess of ammonia, and agitates with ether, which will acquire a purple colour if indirubin be present. For its isolation, Rosenbach treats the fresh urine with lead acetate, heats the filtered liquid to boiling, and adds nitric acid, drop by drop, until a purple colour is produced, carefully avoiding excess of acid. The liquid is then cooled and treated with ammonia till alkaline. The precipitate is filtered off, washed in succession with ammonia, dilute hydrochloric acid, and water, and then dissolved in boiling alcohol. The solution deposits indigo-blue on cooling. It is filtered and the filtrate treated with alcoholic lead acetate, again filtered, and most of the alcohol boiled off. On diluting the residual liquid with water, impure indirubin is precipitated as a brown powder, which, after washing with water, may be purified by crystallisation from chloroform or ether.

[2] Apparently, Méhu's method is intended to apply to ready-formed indigotin and indirubin, but in Michailoff's process it appears to be the indigogens which are precipitated by ammonium sulphate.

hot carbolic acid, to which sufficient glycerin or absolute alcohol has been added to prevent crystallisation on cooling. The colour of a solution of indigo-blue of known strength, prepared in this manner, is compared with that of the urinary pigment.

W. Michailoff (*Jour. Chem. Soc.*, liv. 880) also saturates the acidified urine with finely powdered ammonium sulphate, and then extracts the urobilin by repeated agitations with ethyl acetate (acetic ether). The aqueous layer is next mixed with an equal measure of fuming hydrochloric acid, chloroform added, and then cautiously treated with dilute bromine-water, agitating well between each addition. By presenting the indigo with the solvent when in the nascent state its extraction is said to be very readily and perfectly effected.[1]

Indoxyl-sulphuric acid occurs in very small quantities in normal human urine, Jaffe finding from ·004 to 0·019 gramme in 1500 c.c. of the excretion. Horse's urine contains twenty-three times as much.[2] The proportion in human urine is much increased in certain diseases, such as cholera, typhus, peritonitis, dysentery, and Addison's disease. In obstructive diseases of the small intestine the increase is enormous. The presence of a large amount of indigogens in the urine generally implies that abundant albuminous putrefaction is in progress in some part of the system, these putrefactive products being absorbed and eliminated by the kidneys in the forms of indoxyl-sulphuric acid and its analogue

[1] All the oxidising agents mentioned in the test are liable to destroy the indigo-blue if used in excess. A preferable plan is to employ ferric chloride in presence of hydrochloric acid.

From 25 litres of normal dogs' urine, J. Hoppe-Seyler (*Jour. Chem. Soc.*, xlvi. 1058) isolated 1 gramme of crystallised potassium indoxyl-sulphate and 0·5 gramme of potassium phenyl-sulphate. Neither orthocinnamic acid, orthoamidocinnamic acid, or orthonitrobenzaldehyde, alone or with acetone, produced any increase in the quantity of indigogens excreted.

skatoxyl-sulphuric acid, $C_8H_6C(H_2)N.SO_4H$. The latter body is also found in sweat, and is said to be somewhat more abundant in human urine than the indoxyl-compound. When decomposed by hydrochloric acid or an oxidising agent, it gives a colouring matter usually reddish, but which may possess a marked purple tint.

Traces of compounds of indoxyl and skatoxyl with glycuronic acid (page 37) not improbably exist in normal urine, and their proportions appear to be greatly increased under certain conditions.

APPENDIX.

Weights and Measures.

English Weights.

1 grain, gr.
1 ounce, oz. = 437·5 grains.
1 pound, lb. = 16 ounces = 7000 grains.

English Measures of Capacity.

1 minim	(min.)	= 0·91146 grain-measure.
1 fluid drachm	(fl. drm).	= 60 minims.
1 fluid ounce	(fl. oz.)	= 8 fluid drachms.
1 pint		= 20 fluid ounces.
1 gallon		= 8 pints.

The term *fluid grain* is not official, but is sometimes used to denote the volume occupied by 1 grain weight of distilled water.

Metric System of Weights and Measures.

The basis of the metric system is the length of one ten-millionth of a quadrant of a meridian of the earth's surface, passing through Paris. This length is called a *metre*, and is equal to 39·37079 inches, or very nearly the length of a pendulum vibrating seconds.

The sub-divisions of the metre are the *decimetre* (= 3·937 inches), the *centimetre* (0·3937 inch), and the *millimetre* (= 0·03937 inch).

A *gramme* is the weight of water which occupies, at 4° C., a cube measuring 1 centimetre in the side.

1 milligramme = $\frac{1}{1000}$ part of a gramme, or 0·001 grm.

1 kilogramme = 1000 grammes = the weight of 1000 c.c. or 1 *litre* of water at 4° C.

Relation of English Weights to Metric Weights.

1 grain	=	0·0648	gramme	or	64·8	milligrammes.
2 „	=	0·1296	„	„	129·58	„
3 „	=	0·1944	„	„	194·37	„
4 „	=	0·2592	„	„	259·16	„
5 „	=	0·3239	„	„	323·95	„
6 „	=	0·3887	„	„	388·74	„
7 „	=	0·4535	„	„	453·53	„
8 „	=	0·5183	„	„	518·32	„
9 „	=	0·5831	„	„	583 11	„
10 „	=	0·648	„	„	648·0	„

1 ounce = 437·5 grains = 28·3495 grammes.
1 pound = 7000 grains = 453·5927 „

Relation of Metric Weights to English Weights.

1 milligramme	=	0·01543	grain.
1 gramme	=	15·4323	„
2 „	=	30·8647	„
3 „	=	46·2970	„
4 „	=	61·7294	„
5 „	=	77·1617	„
6 „	=	92·5941	„
7 „	=	108·0264	„
8 „	=	123·4588	„
9 „	=	138·8911	„
10 „	=	154·3234	„

1 kilogramme = 15432·349 grains = 2 lbs. 3 oz. 119·8 grains.

Relation of English Measures to Metric Measures.

1 minim	=	0·05916	cubic centimetre.	
1 fluid drachm	=	3·5495	„	„
1 fluid ounce	=	28·396	„	„
1 pint	=	567·92	„	„
1 gallon	=	4543·36	„	„ , or 4·5434 litres.

Relation of Metric Measures to English Measures.

1 cubic centimetre = 16·9034 minims, 15·432 grain-measures, or 0·28172 fluid drachm.

1 litre = 1000 c.c. = 281·72 fluid drachms or 35·2154 fluid ounces.

Relation of English Measures to English Weights.

1 minim is the measure of 0·91146 grain of water at 60° F.

1 grain-measure ("fluid-grain") is the volume occupied by 1 grain of water at 60° F.

1 fluid drachm is the measure of 54·6875 grains of water.

1 fluid ounce	„	„	437·5	„ or 1 ounce of water.
1 pint	„	„	8750·0	„ or 1·25 pound of water.
1 gallon	„	„	70000·0	„ or 10 pounds „

Conversions.

To convert *grammes per* 100 *c.c.* of liquid into *grains per gallon*, multiply by 700.

To convert *grammes per* 100 *c.c.* of liquid to *grains per fluid ounce*, multiply by 4·375.

To convert *grammes per litre* of liquid into *grains per gallon*, multiply by 70.

To convert *grains per gallon* of liquid into *grammes per litre*, divide by 70.

Relations of Thermometric Degrees.

To convert degrees Fahrenheit into degrees Centigrade, subtract 32, multiply the remainder by 5, and divide the product by 9; or $C = (F - 32) \times 5 \div 9$.

To convert degrees Centigrade into degrees Fahrenheit, multiply by 9, divide by 5 and add 32; or $F. = \frac{9}{5} C + 32$.

Tensions of Aqueous Vapour in Millimetres of Mercury.

°C.	mm.	°C.	mm.	°C.	mm.	°C.	mm.
10 =	9·126	14 =	11·882	18 =	15·351	22 =	19·675
11 =	9·751	15 =	12·677	19 =	16·345	23 =	20·909
12 =	10·421	16 =	13·519	20 =	17·396	24 =	22·211
13 =	11·130	17 =	14·409	21 =	18·509	25 =	23·582

Symbols and Combining Weights of Elements.

Element.	Symbol.	Combining Weight.	Element.	Symbol.	Combining Weight.
Barium,	Ba	137	Magnesium,	Mg	12
Bromine,	Br	80	Manganese,	Mn	55
Calcium,	Ca	40	Mercury,	Hg	200
Carbon,	C	12	Nitrogen,	N	14
Chlorine,	Cl	35·5	Oxygen,	O	16
Copper,	Cu	63	Phosphorus,	P	31
Gold,	Au	196·5	Platinum,	Pt	194
Hydrogen,	H	1	Potassium,	K	39·1
Iodine,	I	127	Silver,	Ag	108
Iron,	Fe	56	Sodium,	Na	23
Lead,	Pb	207	Sulphur,	S	32
Lithium,	Li	7	Zinc,	Zn	65

The combining weights in the above table are in most cases the nearest whole numbers, compared with oxygen as 16. The figures are sufficiently exact for the purposes of this work, but make no pretence to rigidly accurate expression.

Normal and Standard Solutions.

A normal solution is one containing in 1000 cubic centimetres (=1 litre) such an amount of its active constituent as will combine with, replace, or oxidise 1 gramme of hydrogen. Hence normal (expressed $\frac{N}{1}$) solutions of the following substances have the strengths given on next page.

A normal solution may also be described as one containing the hydrogen-equivalent in grammes of the essential substance, with the addition of sufficient distilled water to make up the final volume at 60° F. (=15·5° C.) to 1 litre (=1000 c.c.).

Decinormal solutions are $\frac{1}{10}$ of the strength of normal solutions, and are expressed thus, $\frac{N}{10}$.

Centinormal solutions are $\frac{1}{100}$ of the strength of normal solutions, and are expressed thus, $\frac{N}{100}$.

			Grammes per Litre.
Normal caustic soda	contains	Na	= 23·0
,, ,, ,,	,,	NaOH	= 40·0
,, ,, potash	,,	KOH	= 56·1
,, sodium carbonate	,,	$\frac{Na_2CO_3}{2}$	= 53·0
,, hydrochloric acid	,,	HCl	= 36·5
,, sulphuric acid	,,	$\frac{H_2SO_4}{2}$	= 49·0
,, oxalic acid	,,	$\frac{H_2C_2O_4,2H_2O}{2}$	= 63·0
Decinormal silver nitrate	,,	$\frac{AgNO_3}{10}$	= 17·0
,, potassium permanganate	,,	$\frac{KMnO_4}{5 \times 10}$	= 3·162

Liquor Potassæ and *Liquor Sodæ*, B.P., are approximately normal solutions, the former containing 61·8 grammes of KHO and the latter 43 grammes of NaHO in 1 litre.

Other volumetric solutions of definite strength, but not falling within the above classification, are often very convenient for special purposes. These *standard solutions* are usually designed to measure one particular substance, and are generally prepared so that each cubic centimetre will react with 0·001 or 0·010 gramme of the substance to be measured. Thus, if 4·789 grammes of silver nitrate be dissolved in distilled water, and the solution diluted with distilled water to exactly 1 litre (= 1000 c.c.) at 60° F., each 1 c.c. of the solution will exactly precipitate 0·001 gramme of chlorine.

These standard solutions are simply reciprocals of normal solutions; that is, they have the strength of normal solutions divided by the hydrogen-equivalent of the substance to be measured. Thus, 1 c.c. of *normal* silver nitrate solution precipitates 35·5 milligrammes of chlorine; but if diluted 35·5 times it will give a *standard* solution 1 c.c. of which will precipitate 1 milligramme of chlorine. Such solutions are of great utility in the practical work of the laboratory.

A good example of the application of such a standard solution is afforded by the method employed for determining the proportion of *chlorides in urine*. Direct precipitation of the sample by silver nitrate is not applicable, since much organic matter is thrown down together with the silver chloride. The simplest process generally

applicable is to evaporate 20 c.c. of the urine to dryness in platinum with 3 or 4 grammes of nitre (potassium nitrate) free from chlorides. On gently heating the residue, the organic matter is oxidised by the oxygen of the nitre, and on raising the temperature to incipient redness complete combustion of the carbonaceous matter results, and a perfectly white product is obtained. This, when cold, is treated with hot water, the solution acidulated with nitric acid, a little prepared chalk added, and the whole thoroughly agitated till neutral to litmus. The liquid is then diluted to 100 c.c., and passed through a dry filter. Fifty c.c. of the filtrate (= 10 c.c. of the original urine) should then be placed in a porcelain basin and two drops of a saturated solution of neutral potassium chromate added. A standard solution of silver nitrate containing 4·789 grammes of pure $AgNO_3$ per litre is then gradually added, with constant stirring, until the lemon-yellow colour of the contents of the basin changes to reddish-yellow. This point indicates the conversion of the whole of the chlorides present into white silver chloride, AgCl, and the commencement of the formation of the red silver chromate, Ag_2CrO_4. Every 1 c.c. of the silver solution used represents 0·001 gramme of chlorine in the 10 c.c. of urine employed (= 0·01 gramme per 100 c.c.). Hence if 37 c.c. be required, the urine contains 0·37 per cent. of chlorine, which figure, multiplied by 4·375, equals 1·62 grains of chlorine per fluid ounce.

The chlorine contained in the urine is largely dependent on the quantity of common salt taken with the food, but a portion of it is derived from chlorides of potassium and sodium naturally present in the food. The chlorine found can be calculated into its equivalent of common salt by multiplying it by the factor 1·648 (or, approximately, by dividing it by 0·6). Bromides and iodides, which are not natural constituents of urine but appear after administration of medicines containing them, react like chlorides with silver nitrate. In cases of pneumonia, the chlorides almost entirely disappear from the urine, while the sputum contains an excessive amount.

ERRATUM.

Page 90, line 2, after the word "be" *insert the words* "treated with caustic soda and."

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